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TABLE OF CONTENTS

William Pollock Fraser, 1867–1943 ..... J. H. Craigie 707

A Thecaphora smut on potatoes ..... M. F. Barrus 712

The occurrence of a variant in Rhizoctonia solani ..... L. H. Person 715

Studies on lily virus diseases: the mottle group.
Philip Brierley and Floyd F. Smith 718

Bacterial soft rot of spinach ..... M. A. Smith 747

Experiments with pea seed treatments in Colorado.
J. L. Forsberg, Edward Olson and A. M. Binkley 753

Phytopathological Notes 760

Effect of penicillin on a plant pathogen.
J. G. Brown and Alice M. Boyle

Improved cork-borer method for inoculating trees.
Russell B. Clapper

Lime in the post-arsenical sprays as a means of reducing arsenical injury to peaches ..... Robert H. Daines

A method of inoculating peach seedlings with crown gall without using punctures ..... Clayton O. Smith

The term viruliferous ..... Eubanks Carsner

Resistance of guayule to the root-knot nematode.
Wm. G. Hoyman

PHYTOPATHOLOGY

An International Journal
Official Organ of The American Phytopathological Society

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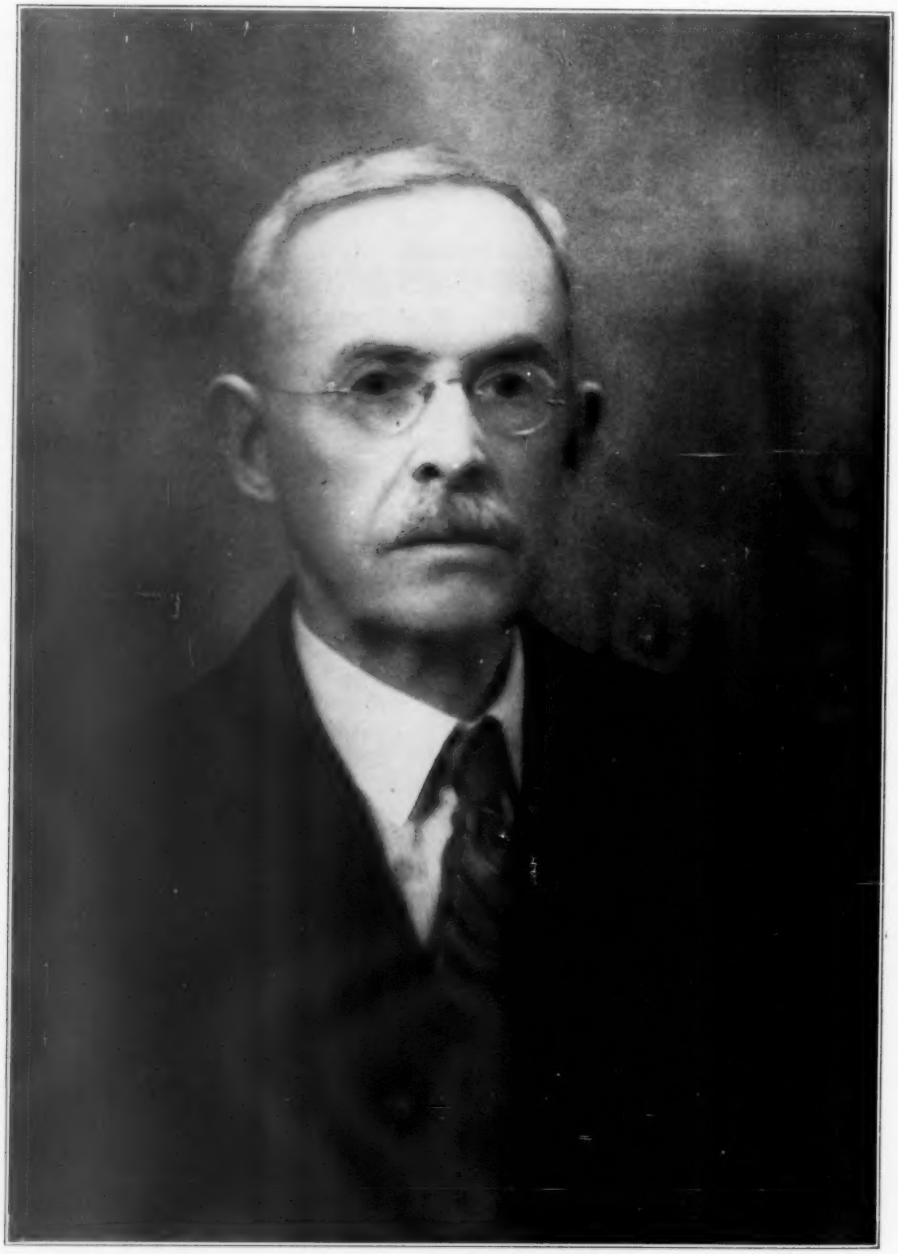
WILLIAM POLLOCK FRASER
1867–1943

J. H. Craigie

William Pollock Fraser, Professor-Emeritus of Biology in the University of Saskatchewan, died at his home in Saskatoon on the morning of November 23, 1943. For several years, he had been afflicted with a heart ailment, which curtailed considerably his activities, but, with the devoted assistance of his wife, he continued his scientific work, at times from a sick bed, until a few days prior to his death. His passing is a pronounced loss to botanical science and to a wide circle of associates, students, and friends who held him in esteem and affection.

Dr. Fraser was born in 1867 at French River, Pictou County, Nova Scotia. He was the son of Alexander and Anna (Pollock) Fraser, and had an older sister and two younger brothers, both of whom died in childhood. He attended the local school—an actual "little red school house." Through the accidental death of his father, he was left in early life to take charge of the home farm. At the age of twenty-one, he yielded to his desire for further education, and, with his mother (his sister having now married), he left the farm and entered New Glasgow High School. He later attended Pictou Academy—from which he graduated in 1896 with senior matriculation—and the Normal School at Truro, N. S. As he had to pay his own way, these years of study were interspersed with years of teaching. In 1899, he enrolled in Dalhousie University, where he studied for two years. He was Principal of Westville High School from 1901 to 1903 and, in the latter year, joined the Staff of Pictou Academy as Instructor in Natural Science. In 1905, he resigned this position to attend Cornell University, from which he received the A.B. degree in 1906. He continued his studies there, and, in 1907, was re-appointed to his former position in Pictou Academy. From Dalhousie University, he received the B.A. degree *ad eundem* in 1907, and the M.A. degree in 1910. In January, 1912, he was appointed to the Staff of Macdonald College as Lecturer in Biology and, in this year, he married Miss Alice Adele McRae. Two years later, he was promoted to Assistant Professor of Biology.

After the disastrous outbreak of stem rust in Western Canada in 1916, the Dominion Department of Agriculture requested him to study the cereal rust situation in that area during the summer of 1917 and of 1918, and, in 1919, appointed him as Officer-in-Charge of the newly established Dominion Laboratory of Plant Pathology at the University of Saskatchewan, Saskatoon, Sask. In the same year, the University of Saskatchewan granted him the M.A. degree *ad eundem* and appointed him, on a part-time basis, as Lecturer in Biology. These appointments he held concurrently until 1925, when he severed his connection with the Department and accepted the position of Professor of Biology in the University. On his retirement in 1937,



WILLIAM POLLOCK FRASER
1867–1943

the University, in recognition of his services, honored him with the LL.D. degree and appointed him Professor-Emeritus of Biology. He was a member of the Associate Committee on Field Crop Diseases of the National Research Council and the Dominion Department of Agriculture. He was also a member of the American Phytopathological Society, a Fellow of the American Association for the Advancement of Science, and a charter member of the Mycological Society of America, of the Canadian Society of Technical Agriculturists, and of the Canadian Phytopathological Society. In the latter Society, he served as Vice-President from 1929 to 1931, as President from 1931 to 1933, and was elected in 1940 to honorary membership.

Dr. Fraser will be remembered for his utter genuineness of character, the keystone of which was his absolute loyalty to truth. He was wholly incapable of subterfuge or dissimulation. Sincere and reserved, he sought out the quiet lanes of life. He craved neither popularity nor distinction. His manner of life was simple, his self-effacement, complete, but his nobility of character and disciplined mind compelled deference and inspired confidence. It was well said of him that he "wore the ornament of a quiet and humble spirit." He was a teacher of wide experience—in elementary, high school, and university work; and just as in his social relations he influenced by example rather than precept, so in his teaching he placed the emphasis more on demonstration than on formal lectures. He was a master of laboratory instruction and was noted for the excellence of his demonstrational material. In research, he worked with his students and associates, as collaborator rather than director, and gave them generously of his time and ability. In return, their regard for him partook of idealization, and they accorded him a respect bordering on reverence.

A born naturalist, Dr. Fraser was familiar with birds and insects, but chose plants as his special field of study. He was an indefatigable collector, and spent most of his spare time in collecting and classifying plants, cryptogams as well as phanerogams, with the object of building up herbaria in the institutions with which he was connected. Although for some years his work was mainly concerned with cereal disease investigations, he retained his interest in the native flora and subsequently devoted an increasing proportion of his time to a study of it. On his retirement in 1937, he undertook the re-organization of the phanerogamic herbarium of the university and also a revision of the list of Saskatchewan plants earlier published by him and Dr. R. C. Russell, neither of which tasks he was able quite to complete.

His direct contribution to plant pathology has been definite and substantial, although at no time was his work entirely restricted to it. He was the pioneer in plant rust research in Canada, his cultural studies of the heteroecious rusts being outstanding. He recognized early the importance of physiological specialization in the cereal rusts and its implications in respect to plant breeding for rust resistance. Likewise, he recognized, and stressed, the urgency of undertaking plant breeding for the production of

rust resistant varieties of cereals. While in charge of rust investigations in Western Canada, he initiated and conducted an annual survey of the physiologic races of stem rust present in that area, and also epidemiological studies on the sources and spread of cereal rusts. By the time he relinquished the active direction of these and related investigations, the general plan of subsequent rust research in Canada was fairly well defined. Other contributions might be mentioned, but probably his greatest contribution to plant pathology, as well as to other fields of botanical science, was indirect, that is to say, through the example of his complete and disinterested devotion to scientific truth and the inspiration he imparted to his students and associates in research.

He was author or co-author of the following publications:

The Erysiphaceae of Pictou County. Bull. Pictou Acad. Sci. Assoc. **1**: 51–58. The Office of The Pictou Advocate, Pictou, N. S. 1909.

Collection of the aecial stage of *Calyptospora columnaris* (Alb. & Schw.) Kühn. Science **30**: 814–815. 1909.

Cultures of some heteroecious rusts. Mycologia **3**: 67–74. 1911.

Cultures of heteroecious rusts. Mycologia **4**: 175–193. 1912.

Further cultures of heteroecious rusts. (Abstr.) Phytopath. **3**: 73. 1913.

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Notes on *Uredinopsis mirabilis* and other rusts. Mycologia **6**: 25–28. 1914.

Notes on some plant diseases of 1913. Ann. Rept. Quebec Soc. Protect. Plants **6**: 45–50. 1914.

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Methods for controlling smut. Public Service Monthly (Sask.) **8**: 14. May, 1920.

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List of the flowering plants, ferns and fern allies of Saskatchewan. (With R. C. Russell.) 46 pp. University of Saskatchewan, Saskatoon. 1937.
Additions to the list of flowering plants of Saskatchewan. (With R. C. Russell.) 7 pp. University of Saskatchewan, Saskatoon (mimeographed). 1938.
The fungi of Manitoba and Saskatchewan. (With G. R. Bisby, A. H. R. Buller, J. Dearness and R. C. Russell.) 189 pp. National Research Council, Ottawa. 1938.
Notes on the Cyperaceae of Saskatchewan. I. Scirpus. The Can. Field-Naturalist **54**: 100–101. 1940.
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A THECAPHORA SMUT ON POTATOES

M. F. Barrus

(Accepted for publication March 25, 1944)

Barrus and Muller[1] described a disease of potato tubers, known locally as *buba* disease, occurring in the Andes Mountains of Venezuela on Andean varieties (*Solanum andigenum* Juz. et Buk.). It is characterized by the presence of warts or lumps on the surface and by numerous brown specks throughout the flesh. These specks prove to be cavities filled with rusty

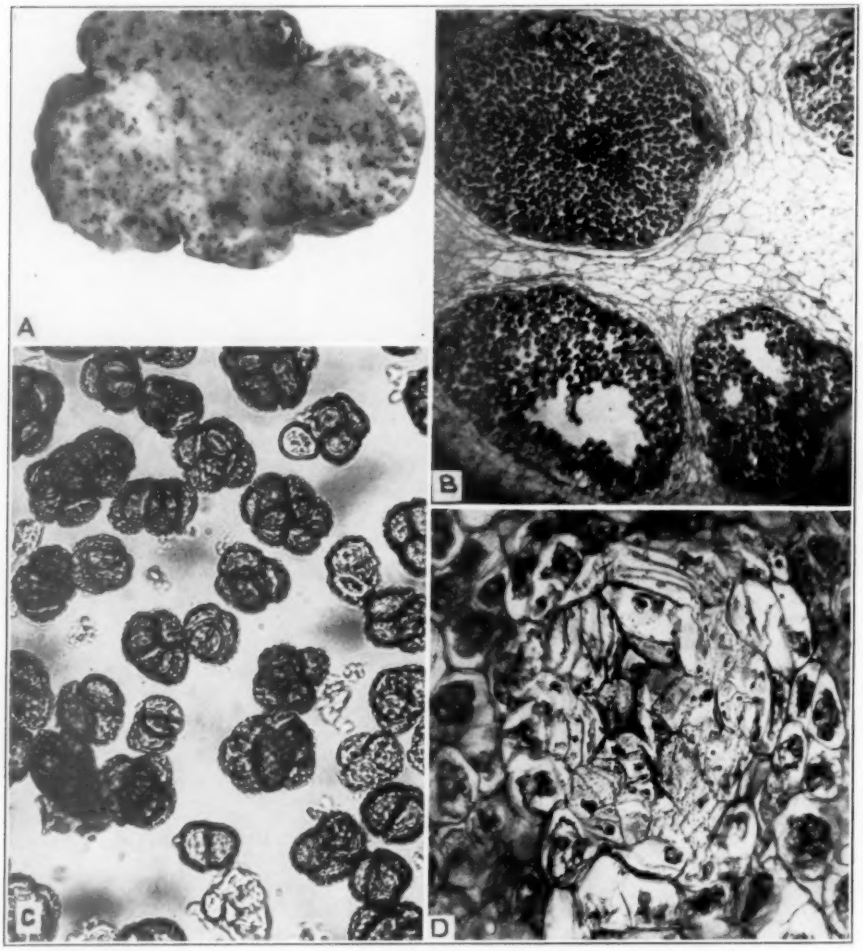

Fig. 1. *Thecaphora solani* on *Solanum andigenum*. A. Longisection of a tuber showing lobed surface and internal specking, ×1.2. B. Sori of smut in flesh, ×31.7. C. Sporeballs, ×328.5. D. Young sorus showing phellem, ×166.25. Photographed by W. R. Fisher. B and D from stained slides made by Ruby Rice.

[1] Barrus, M. F., and A. S. Muller. An Andean disease of potatoes. Phytopath. 33: 1086–1089. 1943.

brown sporeballs consisting of 1 to 8 spores prominently and abundantly verrucose on the exposed surfaces (Fig. 1, A, B, and C).

Since that article was prepared, I have had an opportunity to examine other material sent by J. Camero Zamora from the same locality as well as material sent by John A. Stevenson of the United States Department of Agriculture from Peru, from Ecuador, and from the original collection by Chardon in Venezuela. Attempts have been made to germinate the spores, but thus far without success. It was first thought impossible to determine the pathogen without observing the type of spore germination but, after

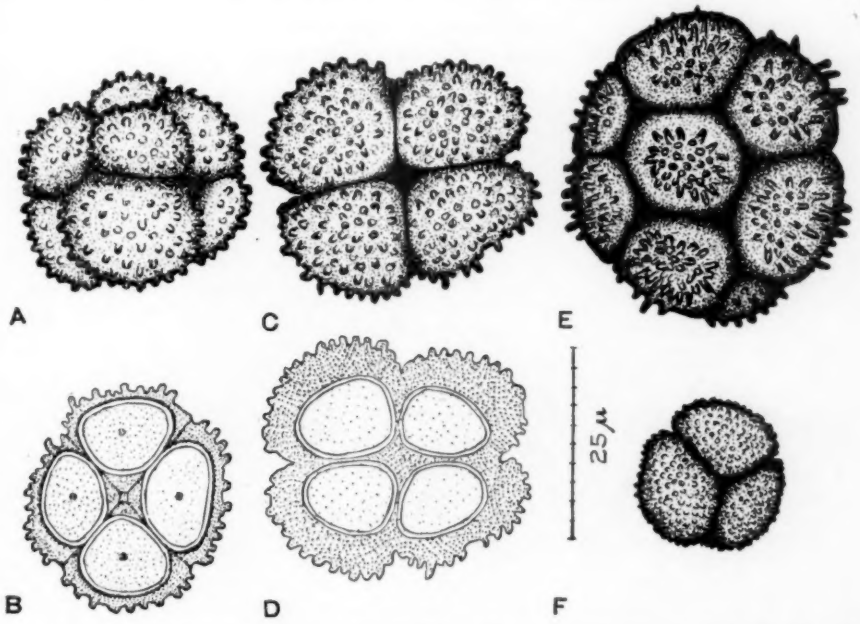

FIG. 2. Sporeballs of four species of *Thecaphora* showing similarity in shape, size, and character of outer wall. Drawn to the same scale with aid of camera lucida by Carlos Garces. Dry spores mounted in Shear's mounting fluid, except B which is from a stained tissue section made by Ruby Rice. A. *T. solani* Bar., CUPP 33247; B. *T. solani*, CUPP 29427; C. *T. pustulata* Clin. CUPP, Fungi of Puerto Rico 458; D. *T. pustulata*, optical section of C; E. *T. deformans* D. et M., Zillig Ust. Eur. 84; F. *T. trailii* Cke. Krieg. Fung. Sax. 1451.

making a comparison of the sporeballs of this fungus with those of other species of smut fungi, I have come to the conclusion that it is a new species of *Thecaphora*. This conclusion is based on the similarity to several other species of this genus in size, shape, and color of the sporeballs, the number of spores composing them, and the thick verrucose wall on their exposed surfaces (Fig. 2). The spores of these other species have also been germinated with difficulty or not at all.

The sporeballs of this new species of *Thecaphora* in the several collections are very similar but the average measurements differ slightly. The spores have a definite inner wall up to 1 μ thick. The spore measurements given

here do not include the outer wall because of the difficulty of determining its exact limits. This varies in thickness from about 0.5 μ or less on the side adjacent to other spores in the ball to as much as 1.5 to 4 μ on the exposed surfaces.

As Barrus and Muller described the disease and the fructifications of the pathogen rather completely, there is given here only a description of the fungus sufficient to establish its position. In naming it, consideration is given to the possibility of its being classified elsewhere when more information regarding its development is available.

Thecaphora solani n. sp.

Sori numerous, embedded in the cortex and pith of warty, lobed, or otherwise misshapen tubers; subcircular, oval or irregular in cross section; up to 1 mm. long; containing rusty to dark-brown spore masses; occasionally two or three sori adjoining each other; surrounded when young by phellem (Fig. 1, D): sporeballs yellowish to rusty brown, dusty after exposure to air, mostly subspheric to ovoid, 12–48 × 12–35 μ, consisting of 2 to 8 adherent spores (usually 3 to 6 and occasionally a single one); spores subspheric to ovoid or somewhat angular in optical section, 7.5–20 × 8–17.5 μ, with contiguous walls smooth and the free wall rounded, thick, and abundantly verrucose.

Habitat: In tubers of an Andean variety of *Solanum andigenum* Juz. et Buk.

Type: CUPP[2] 29427, collected by Barrus and Muller at Mucuchíes, State of Mérida, Venezuela, on November 19, 1939. Type material has also been distributed to the following herbaria: Mycological Collections, Bureau of Plant Industry, U. S. Department of Agriculture, Beltsville, Maryland; New York Botanical Garden, New York City; Farlow Herbarium, Cambridge, Massachusetts; Pennsylvania State College of Agriculture, State College, Pennsylvania; University of Michigan, Ann Arbor, Michigan; University of Toronto, Toronto, Canada; University of Wisconsin, Madison, Wisconsin; Missouri Botanical Garden, St. Louis, Missouri; Royal Botanic Gardens, Kew, England; and British Museum of Natural History, London, England.

Distribution: Andes Mountains at elevations of 2500 to 3300 meters in Venezuela, Ecuador, and Peru.

Other Material Examined: BPI[2] 71386 and CUPP 33244, collected by Carlos E. Chardon near Mérida, Venezuela, in August, 1932, and sent by him to the United States Department of Agriculture, Bureau of Plant Quarantine, Washington, D. C.; BPI 71388 and CUPP 33245, collected by J. Soukup, Puno, Peru, in April, 1940; BPI 71387 and CUPP 33246, collected by Luis Rodriguez Lz. at Alausí, Ecuador, in January, 1943; and CUPP 33247, collected by Carlos Gonzalo Salas at Mucuchíes, Venezuela, on April 28, 1934, at the request of J. Camero Zamora of the Agricultural Experiment Station, El Valle, Venezuela.

CORNELL UNIVERSITY,
ITHACA, NEW YORK.

[2] CUPP refers to the herbarium of Cornell University, Department of Plant Pathology, Ithaca, N. Y., and BPI to the Mycological Collections, Bureau of Plant Industry, U. S. Department of Agriculture, Beltsville, Maryland.

THE OCCURRENCE OF A VARIANT IN RHIZOCTONIA SOLANI[1]

L. H. Person

(Accepted for publication February 17, 1944)

In the course of studies of various isolates of *Rhizoctonia solani* Kühn, a sector was found in a culture, isolated originally from a lesion on a bean stem (Fig. 1). Transfers from the sector, when compared with the original culture, were found to be distinct, the mycelium being somewhat lighter in color and with more aerial growth. As reports of variants in this fungus seem relatively rare, being confined to ones by Ullstrup[2] and LeClerg,[3] a

Fig. 1. Parent culture of *Rhizoctonia solani* and sector variant.

comparative study was made of the temperature relations and pathogenicity of the parent culture and variant.

It is realized that the mode of origin of the sector may be questioned, as the original culture was a tissue isolate and may not have come from a single hypha. This, however, seems improbable, as the sector did not appear until after the original culture had been studied for 3 years under a variety of conditions.

TEMPERATURE RELATIONS

The radial growth of the original culture and variant were compared on bean-pod agar at temperatures between 15° and 34° C. The results shown in table 1 indicate that the parent culture grew more rapidly than the variant at 20° and 24°, about the same at 29°, and somewhat more slowly at 34°.

[1] Excerpt from a thesis presented in partial fulfillment of the requirements for the degree of Doctor of Philosophy, granted by the University of Minnesota, August, 1937.

[2] Ullstrup, Arnold J. Leaf blight of China Aster caused by *Rhizoctonia solani*. Phytopath. 26: 981–990. 1936.

[3] LeClerg, E. L. Studies on a cultural variant of *Rhizoctonia solani*. Phytopath. 29: 267–274. 1939.

TABLE 1.—*Growth of Rhizoctonia culture and its variant as shown by diameter of colony in mm. after 50 hours (average of 2 tests)*

Culture	15° C.	20° C.	24° C.	29° C.	34° C.
Original culture	25	59	88	83	65
Variant	24	47	76	83	69

PATHOGENICITY

Inoculations were made in sterilized soil in the greenhouse to compare the pathogenicity of the parent culture and variant. The method was the same as given elsewhere.[4] To determine the pathogenicity, the stand in per cent and the severity of stem lesions were used. To measure the severity of the stem lesions caused by a culture, each plant was placed in 1 of 5 classes ranging from 0 to 4, zero indicating no stem lesions and 4 indicating that the plants were killed. The degree of infection was then calculated as the average of the value of the stem lesions on all the plants inoculated with a culture. The results of 3 tests (Table 2) showed that the variant was less pathogenic than the parent culture.

TABLE 2.—*Comparative pathogenicity of original culture and variant to beans (average of 3 tests of 100 seeds each)*

Inoculum	Planted in inoculated soil		Soil inoculated after emergence of plants
	Per cent stand	Degree of infection	Degree of infection
Original culture	52	2.21	3.13
Variant	69	1.01	1.69
Control	84	0.00	0.00

Since a difference between the original culture and variant occurred in pathogenicity on beans, 2 more tests were made, which also included soybeans, cowpeas, and English peas. The results (Table 3) indicate the variant was less pathogenic on each host than the original culture.

TABLE 3.—*Comparative pathogenicity of original culture and variant on beans, soybeans, cowpeas, and English peas (average of 2 tests of 100 seeds each)*

Inoculum	Stand in per cent			
	Beans	Soybeans	Cowpeas	English peas
Original culture	63	1	40	0
Variant	78	36	76	81
Control	92	56	94	94

[4] Person, L. H. Parasitism of *Rhizoctonia solani* on beans. In manuscript form.

SUMMARY

A sector variant, which occurred in an isolate of *Rhizoctonia solani* from snapbean, was compared with the original culture in growth rate at different temperatures and in pathogenicity. The variant differed in growth rate at certain temperatures and was less pathogenic than the original culture on beans, soybeans, cowpeas, and English peas.

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STUDIES ON LILY VIRUS DISEASES: THE MOTTLE GROUP

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Lily mosaic as reported by Guterman (6, 7) was chiefly a mottling disease, but the necrotic fleck symptoms in Easter lily (*Lilium longiflorum* Thunb.) were included without recognition of their separate etiology. McWhorter's reports (12, 14) of tulip breaking induced by mechanical inoculation with sap of various lilies encouraged the use of tulips (*Tulipa*

FIG. 1. Virulent-mottle (VCM) symptoms in Easter lily seedling. Note narrowed leaves and leafless interval in stem with normal leaves above and below. Inoculated Feb. 17, 1943. Photographed in 5-inch pot July 14, 1943, by Mead.

gesneriana L.) as test plants for lily viruses both at the Oregon Experiment Station and at the Plant Industry Station, Beltsville, Maryland. As shown later (3) *Lilium formosanum* Stapf is also a sensitive test plant for most lily viruses of the mottle type. Studies on the mottle viruses as well as on the necrotic-fleck complex (5) indicate that the mottle viruses are of major importance to some garden lilies, but of lesser importance in commercial

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Easter lily culture. This paper reports data on properties, host range, and vector relations of 3 lily-mottle viruses assigned to the tulip virus group, with comparative data on McWhorter's (15) Tulip viruses 1 and 2.

Data on physical properties, host range, and vector relations of the strong mottle virus of Easter lily (3) (CM or coarse mottle in our notation) have been accumulated since 1937. In 1939 McWhorter supplied samples of Tulip virus 1 and Tulip virus 2 (15) in tulips, and of a latent virus of lily (14) in *Lilium tigrinum* Ker-Gawl. A more virulent virus (VCM or virulent coarse mottle) appeared in 1942 in Creole Easter lilies (Fig. 1)

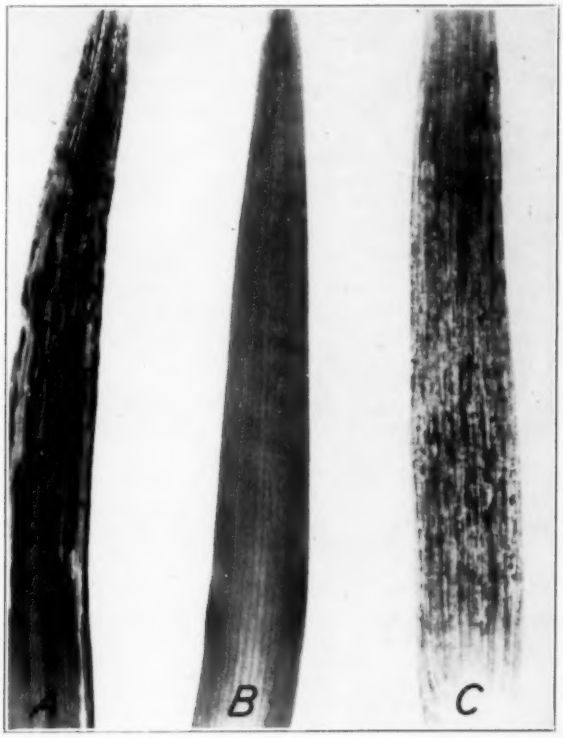

FIG. 2. Symptoms in *Ornithogalum thyrsoides* of (A) lily-virulent-mottle virus (VCM), and (C) Ornithogalum-mosaic virus, in comparison with (B) healthy control leaf. (A) and (C) transferred by *Myzus persicae*. $\times \frac{1}{2}$.

previously affected with the strong (coarse) mottle (CM). These 5 virus cultures were then compared in selected test plants, and parallel determinations of properties were made in *L. formosanum* to decide whether they must be considered distinct or if they may be interpreted as strains of one virus.

SOURCE OF VIRUS CULTURES

CM (coarse mottle), the common mottling virus of Louisiana Creole Easter lilies, isolated from one of these in 1937, passed through tulip, and repeatedly subcultured in *Lilium longiflorum* and *L. formosanum* seedlings.

VCM (virulent coarse mottle), transferred from a naturally affected Creole Easter lily to *Ornithogalum thyrsoides* Jacq. (Fig. 2) and thence to Easter lily seedlings by *Myzus persicae* Sulz., then subcultured mechanically in Easter lily and *Lilium formosanum* seedlings.

LT, a latent virus isolated from *Lilium tigrinum* furnished by McWhorter in 1939, and subcultured in *L. tigrinum* and *L. formosanum*. Inasmuch as this LT culture produces predominantly dark breaks in tulips, it should not be construed as typical of the latent virus of lily reported by McWhorter (14).

TABLE 1.—*Symptoms of 5 mottle viruses in 4 selected test plants*

Virus	Easter lily	*Lilium formosanum*	*Lilium tigrinum*	Clara Butt tulip
VCM	Coarse mottling, striping, twisting, and narrowing of leaves; occasionally flower distortion.	Yellowing, dwarfing, fine green islands on yellowish green ground.	Gray surface etch followed by general yellowing and death.	Mild leaf mottling; mixed type flower break, bleaching predominant.
CM	Coarse mottling only.	"	"	Strong yellow leaf mottling; mixed type flower break, bleaching predominant.
LT	Symptomless.	No yellowing; no dwarfing; upright habit; green island mottling.	Fine green mottling, soon masked.	Barely perceptible leaf mottling; mixed type flower break, intensification predominant.
TV1	Symptomless.	No yellowing; no dwarfing; upright habit; sparse and fine green islands.	Green mottling, soon masked.	Mild leaf mottling; mixed type flower break, bleaching predominant.
TV2	Symptomless.	No yellowing; no dwarfing; upright habit; coarse dark and light green mottling.	Gray surface etch only.	Mild leaf mottling; mixed type flower break, intensification predominant.

TV1, tulip virus 1 (15) isolated from Clara Butt tulip furnished by McWhorter in 1939, and subcultured in tulip and *Lilium formosanum*.

TV2, tulip virus 2 (15) isolated from Farncombe Sanders tulip furnished by McWhorter in 1939, and subcultured in tulip and *Lilium formosanum*.

GENERAL CHARACTERIZATION OF THE FIVE MOTTLE VIRUSES STUDIED

The principal test reactions by which these 5 viruses are distinguished are shown in table 1. The Easter lily mottle virus (CM) is characterized by coarse mottling without leaf distortion in this species (Fig. 3), by bleaching or color removing breaks in tulips (Fig. 4), and by its killing effect in tiger lilies (Fig. 5, A). This virus is apparently universal in Creole Easter

lilies. Some lines of this variety are typically symptomless, *e.g.*, the Norwood clon from Louisiana and a stock indistinguishable from Creole received from a Florida source. These symptomless stocks give the characteristic test reactions for CM in *Lilium formosanum* (Figs. 5, B, and 6, B), *L. tigrinum* (Figs. 5, A, and 7, A), and tulip (Fig. 4).

Virulent mottle virus (VCM) produces effects similar to CM in all test plants but two. This virus alone of the mottle group has been transmitted to *Ornithogalum thyrsoides,* inducing a conspicuous coarse green mottling (Fig. 2), distinct from the fine green mottling of Ornithogalum mosaic (18). In Easter lily, VCM produces yellow stripes as well as mottling (Figs. 1, 3), and also various leaf deformations. Some leaves are curved laterally, others

FIG. 3. Leaf symptoms in Easter lily. Left to right VCM, CM, LT, TV1, TV2. The last 3 symptomless, proved by indexing. Photographed by Mead.

are greatly reduced in width, and some are filiform. Occasionally Easter lily stems are bare of leaves for several inches with mottled leaves of nearly normal form above and below. When a zone of strong symptom expression occurs at bud stage, the flowers are variously deformed, with curled and narrowed perianth segments. VCM was first recognized in 1942, in several commercial fields in Louisiana, and in several greenhouses at Beltsville, Maryland, always in Easter lily stocks of previous CM history. Circumstances suggested a variant of CM rather than a new virus contaminant, and data subsequently obtained support this assumption. Survival of VCM from season to season in Creole lilies grown under glass has been low, 1 in 17 in one lot, the remainder reverting to CM symptoms.

A latent virus (LT) in tiger lily, obtained from McWhorter (14), produces dark intensifying flower breaks in tulip (Fig. 4), thus differing sharply from TV1 with which it agrees rather closely in other test-plant

reactions (Figs. 3, 5, A, B, 6, 7, 8). In McWhorter's original account (14), latent viruses transferred from Easter lily, *Lilium candidum* L., and *L.*

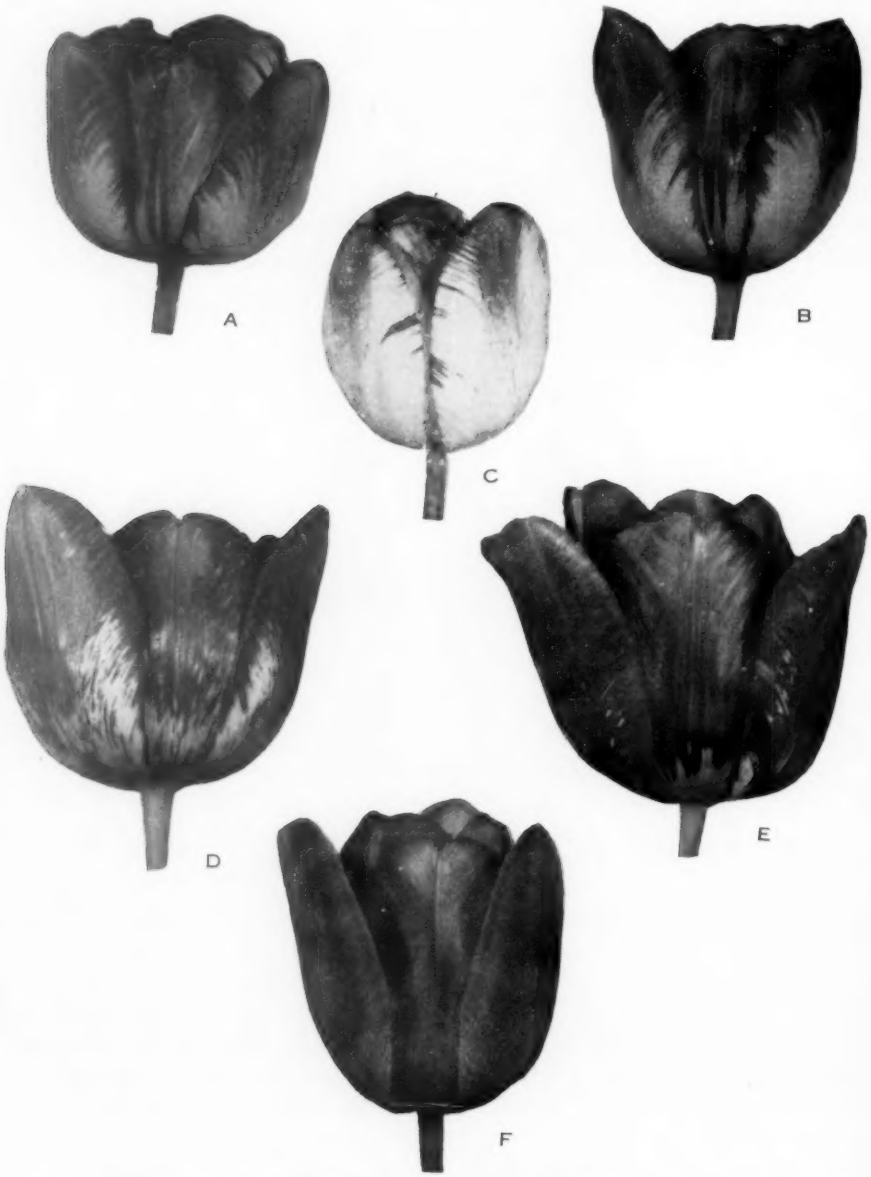

FIG. 4. Symptoms of tulip-breaking viruses in flowers of Clara Butt tulips. A, VCM; B, CM; C, TV1; D, LT; E, TV2; F, normal flower. A, D, E transferred by *Myzus persicae;* B, C transferred mechanically. Photographed February, 1943, current season symptoms.

tigrinum were considered identical with TV1, but he has later (16) pointed out cytological symptoms that distinguish some of them. The bleaching-

type breaks in tulips reported characteristic of "lily latent virus" in his first report (14), and pointing to identity with TV1, often result from inoculations from Easter lily and *L. candidum* into tulips, but not from this

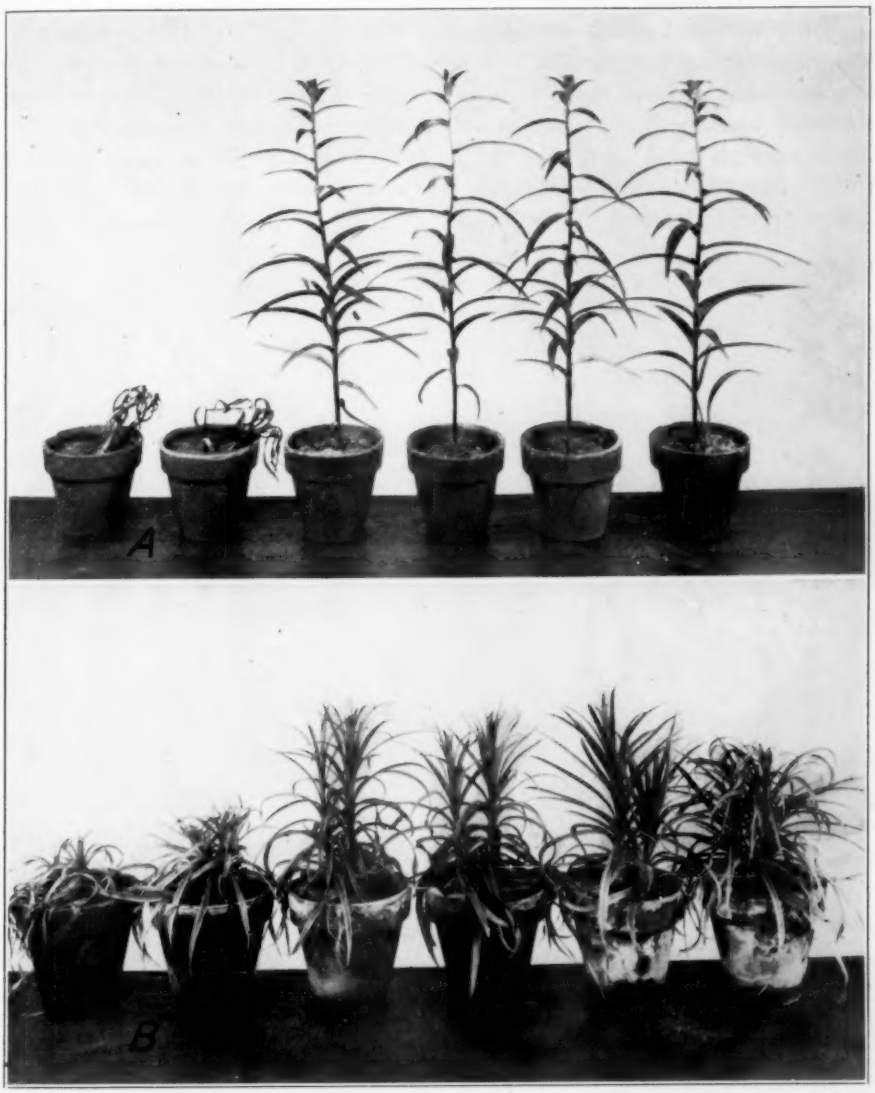

FIG. 5. Reactions of tulip-breaking viruses in (A) *Lilium tigrinum* in 4-inch pots inoculated Mar. 24, 1943, and (B) *L. formosanum* in 5-inch pots inoculated Mar. 25, 1943. Left to right VCM, CM, LT, TV1, TV2, control. Note VCM and CM are fatal to *L. tigrinum*, cause dwarfing in *L. formosanum*. Photographed May 8, 1943, by Mead.

L. tigrinum source. The virus we term LT in this paper, therefore, may be regarded as a sample of latent virus from *L. tigrinum* but not as type material of McWhorter's latent virus of lily (14). LT or a similar virus

is common but not universal in tiger lilies, stocks free from it having come to our hands from Maryland, New York, Oregon, and Vermont. Affected tiger lilies usually show a well-defined, mild, green leaf mottling in early stages of development, but become symptomless before flowering.

Tulip viruses 1 and 2 were segregated by McWhorter (15) as bleaching and intensifying components, respectively, which in mixture produce the average break or Rembrandt type in various Darwin tulips. These viruses, although uncommon in lilies, are obviously more or less closely allied to the lily-mottle group. Tulip virus 1 (TV1) differs from Tulip virus 2 (TV2) and from LT in producing bleaching breaks in tulip (Fig. 4) and differs from CM and VCM in failing to produce symptoms in Easter lily

FIG. 6. Symptom expression of mottle viruses in leaves of *Lilium formosanum:* A, VCM; B, CM; C, LT; D, TV1; E, TV2; F, control. Photographed 36 days after inoculation.

(Fig. 3). TV2, a color-adding or intensifying type in tulip (Fig. 4), differs from the other viruses, except LT, here discussed in this respect. It is distinct from LT in the detailed symptoms expressed in both *L. tigrinum* (Fig. 7) and *L. formosanum* (Fig. 6).

The symptoms expressed in *Lilium formosanum* (Figs. 5, B, 6) fail to distinguish VCM from CM, but are recognizably different for the other 3 viruses studied. Inasmuch as VCM is known only from Easter lily, in which it is distinctive, *L. formosanum* is a valuable test plant for preliminary classification of unknowns. The CM and LT types encountered in lilies yield distinctive reactions in all the test plants listed (Figs. 3, 4, 5, A, B, 6, 7, 8). The sharpest distinction between these 2 virus types is found in *L. tigrinum* in which LT is symptomless after mild mottling in early growth, but which CM kills outright in 6 to 8 weeks (Figs. 9, 10, 5, A).

FIG. 7. Symptom expression of mottle viruses in leaves of *Lilium tigrinum:* A, lily-mottle virus CM (similar reaction from VCM not shown); B, LT; C, TV1; D, TV2.

Some virus cultures obtained in earlier indexing (3) were retained for a time and tested on Easter lily and tulip as well as *Lilium formosanum,*

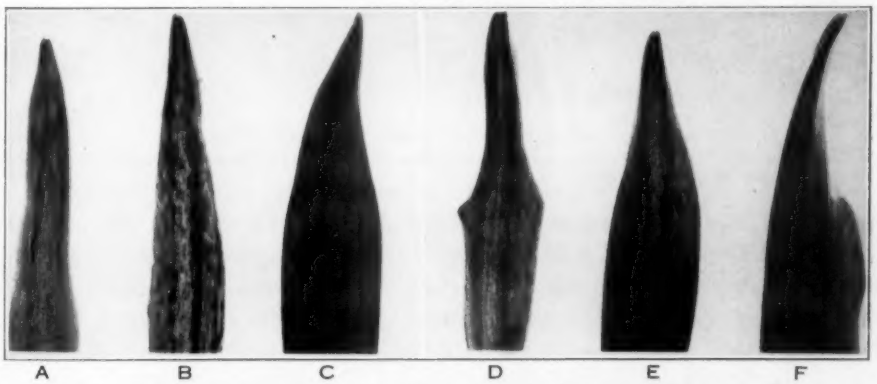

FIG. 8. Symptom expression of mottle viruses in leaves of Clara Butt tulip: A, VCM; B, CM; C, LT; D, TV1; E, TV2, in comparison with (F) control. A, B, C, E transferred by *Myzus persicae,* D transferred mechanically. Photographed by transmitted light.

but the *L. tigrinum* test was not then in use. The reactions of these 3 test plants indicate the presence of CM in Easter lily from several sources including occasional plants from Oregon, in some stocks of *L. candidum* from Oregon, in one *L. sargentiae* Wilson from Maryland, and in one *L. superbum* L. from New York. Reactions characteristic of LT were found for cultures from *L. auratum* Lindl. of Japanese origin, from some *L. tigrinum, L.*

FIG. 9. Initial symptoms of CM photographed 15 days after inoculation into previously healthy *Lilium tigrinum* (in 6-inch pot). Gray etch in leaves immediately above those showing rubbing injury.

candidum, and *L. superbum* plants from New York, and from some Easter lilies from Oregon. Some isolates fail to fit the categories of table 1, which was prepared to distinguish the 5 cultures herein discussed, but which is not intended as a key to all mottle viruses affecting lily and tulip.

HOST RANGE OF THE EASTER LILY MOTTLE VIRUS (CM)

CM, which is readily transmissible by sap with the aid of carborundum, was tested by this method on a wide range of plants. No hosts were found

among 79 species of dicotyledonous plants representing 66 genera and 29 families, and including many of the common vegetable and ornamental plants. Subinoculations to *Lilium formosanum* from many of these plants were also uniformly negative. It is therefore believed unlikely that this virus is capable of infecting dicotyledonous plants.

A further survey of representative monocotyledons revealed no hosts for CM virus outside the Liliaceae, and few in this group. The following monocots were tested without result. AMARYLLIDACEAE: *Amaryllis* hybrids,

FIG. 10. Symptoms of CM in *Lilium tigrinum* (A) in comparison with control (B). (A) inoculated with LT in June, 1942, followed by CM in March, 1943. Photographed (in 5-inch pots) by Mead 45 days after CM was superimposed on LT. No protection was afforded by LT.

Narcissus pseudonarcissus L., *Zephyranthes* sp.; ARACEAE: *Philodendron* sp., *Zantedeschia aethiopica* Spreng.; CANNACEAE: *Canna generalis* Hort.; COMMELINACEAE: *Commelina coelestis* Willd., *C. communis* L., *Rhoeo discolor* Hance; DIOSCOREACEAE: *Dioscorea alata* L.; GRAMINEAE: *Avena sativa* L., *Saccharum officinarum* L., *Secale cereale* L., *Zea mays* L.; IRIDACEAE: *Moraea iridioides* L., *Tritonia crocata* Ker-Gawl.; LILIACEAE: *Agapanthus africanus* (L.) Hoffmg., *Allium cepa* L., *A. cernuum* Roth., *Aloe* sp., *Asparagus sprengleri* Regel, *Asphodeline lutea* Reichb., *Brodiaea uniflora* Baker, *Dracaena fragrans* Ker-Gawl., *D. sanderiana* Hort., *Gloriosa rothschildiana*

O'Brien, *G. superba* L., *Hyacinthus orientalis* L., *Kniphofia tucki* Baker, *Muscari polyanthum* Boiss., *Nothoscordum fragrans* Kunth., *Ophiopogon jaburan* Lodd., *Ornithogalum thyrsoides* Jacq., *Scilla hispanica* Mill., *Tricyrtis hirta* Hook., *Yucca flaccida* Haw.; MARANTACEAE: *Maranta bicolor* Ker.; MUSACEAE: *Musa cavendishii* Lamb.; ZINGIBERACEAE: *Hedychium coronarium* Koenig.

The established hosts of the CM virus are listed in table 2. In addition to species of *Lilium*, *Calochortus* sp., *Fritillaria pudica* Spreng., and *Zygadenus fremontii* Torr. have been shown to be susceptible. No symptoms were recognized in the two first named, but the virus was recovered. *Zygadenus fremontii* developed a fine green mottling resembling the pattern of iris

TABLE 2.—*Proved host plants of the Easter lily mottle virus (CM)*

Plants inoculated	No. of trials	Plants affected[a]	Symptoms	Subinoculation
Calochortus sp.	4	?/25	None	+
Fritillaria pudica Spreng.	2	?/10	"	+
Lilium dauricum Ker-Gawl.	1	2/2	Mottling, yellowing, death	
L. davidi Duchartre var. *Willmottiae*	1	1/4	Mottling	+
L. elegans Thunb.	1	3/3	Necrotic spots, yellowing, death	
L. formosanum Stapf	35	242/257	Mottling	+
L. leucanthum Baker	1	3/5	"	+
L. longiflorum Thunb.	28	195/275	"	+
L. superbum L.	1	2/5	"	+
L. tigrinum Ker-Gawl.	10	46/48	Necrotic spots, yellowing, death	+
L. umbellatum Hort.	1	3/3	Mottling, yellowing, death	
Tulipa gesneriana L.	39	264/428	Mottling, flower breaks	+
Zygadenus fremontii Torr.	3	5/11	Mottling	+

[a] Number plants affected over number exposed.

mosaic in bulbous iris. None of these plants were found naturally infected on receipt from plant dealers. Guterman (6) found *Fritillaria camtschatcensis* L. naturally affected with a mosaic only when grown in a nursery near affected lilies.

Comparatively few species of *Lilium* have been adequately tested for susceptibility because of the difficulty of obtaining vigorous stocks of known freedom from virus. Inoculation with CM resulted in mottling in seedlings of *L. davidi* var. *Willmottiae*, *L. leucanthum*, and *L. superbum*, and the virus was recovered from each of these. *Lilium dauricum*, *L. elegans*, *L. tigrinum* (Figs. 9, 10, 5, A), and *L. umbellatum* seedlings were killed by the virus. In parallel tests mechanical inoculation produced no infection in *L. davidi*, *L. henryi* Baker, *L. pardalinum* Kellogg, or *L. regale* Wilson seedlings, and transfers of *Aphis gossypii* Glover led to no infection in *L. humboldti* Roezl. and Leicht. var. *magnificum* Purdy, or in *L. parvum* Kellogg. The virus was not recovered from these species, but single trials even under conditions

favorable for infection of other species are not considered conclusive. As stated above, the CM type has been recovered from naturally affected *L. candidum*. It is to be expected that many other species of *Lilium* will prove susceptible, but this is the complete host list thus far proved for the CM virus.

VCM is mechanically transmissible to and from the test species listed in table 1. It has been so transmitted to *Zygadenus fremontii*, inducing symptoms indistinguishable from those of CM, but failed to infect *Calochortus* sp. in a single trial. Other host species of CM (Table 2) have not been inoculated with VCM. This virus alone of the group discussed herein has been transmitted to *Ornithogalum thyrsoides* by *Myzus persicae* (Fig. 2). Mechanical transfer from Easter lily to *Ornithogalum* failed, but VCM established in *Ornithogalum* by *M. persicae* was mechanically transferred to *Ornithogalum*, to Easter lily, and to *L. formosanum*. *Myzus persicae*, an efficient vector of VCM, failed to transfer this virus from Easter lily to Imperator iris (*Iris filifolia* Boiss.), to *Allium cernuum* Roth., to California Red onion, to *Allium fistulosum* L., or to *A. porrum* L. The known host range of VCM thus coincides with that of CM with 2 discrepancies, namely, the failure of VCM to infect *Calochortus* in a single trial, and the positive infection of *Ornithogalum thyrsoides*.

LT failed to infect either *Zygadenus fremontii* or *Calochortus* sp. in single trials. It may occur in additional species of lilies in nature, but only the species listed in table 1 have been experimentally infected. Similarly TV1 and TV2 have been studied here only in the 4 test plants listed in table 1. McWhorter (13) reports negative tests on onion, iris, narcissus, *Brodiaea*, and *Camassia*. The 2 tulip viruses have proved difficult to transmit from *Lilium formosanum* to Easter lily by sap, but this transfer has been proved by return inoculations from some of these symptomless Easter lilies to *L. formosanum*.

PROPERTIES OF THE EASTER LILY MOTTLE VIRUS (CM)

Preliminary data on the physical properties of CM were collected soon after this virus was recognized. It was soon evident that *Lilium formosanum* was a favorable source and test plant for such studies, but heavy demands on available stocks of this species as a test subject for subinoculation and indexing made it impracticable to complete property studies at once. Some determinations were therefore made with Easter lily as the source plant and Clara Butt tulips as test subjects. Sap for such tests was extracted in mortars and strained through cloth but not further cleared or purified. In thermal inactivation tests, 2-cc. samples of undiluted juice were pipetted into standard test tubes sold for this purpose, and heated in a De-Kotinsky water bath. In aging experiments undiluted juice was stored at 18° C. in open containers and 2-cc. samples tested for activity at stated intervals. For drying tests 5-cm. squares of cheesecloth were saturated with freshly extracted juice and suspended in shade at room temperature. After

stated intervals these squares were soaked in 10 cc. of tap water and used as inoculation pads.

Data on properties of CM virus are shown in table 3. The thermal inactivation point apparently lies between 60° and 65° C. and the dilution end point between 10^{-3} and 10^{-4}. The virus withstood aging 1 day but not 2 days, and was inactive after drying 1 day on cloth squares. Consistently lower tolerances were found when mottled Easter lily seedlings served as source of virus and forced Clara Butt tulips as test plants, indicating that this combination of species is poorly adapted to property studies. Norwood Creole Easter lily, a symptomless clon carrying the CM virus as shown by the reaction of tulip, *Lilium formosanum,* and *L. tigrinum,* yielded even lower readings, suggesting that the concentration of CM in this clon may be very low.

TABLE 3.—*Properties of the Easter lily mottle virus (CM)*

PROPERTIES OF FIVE VIRUSES OF THE TULIP GROUP

Parallel determination of properties of the 5 viruses were made in *Lilium formosanum,* the same species serving as source of virus. In general the source material was taken from plants about 3 weeks after inoculation when symptoms were well expressed and abundant leaf material was available. The procedures were as described above. In each experiment the source material, test plants, and manipulations were as uniform as possible for the 5 viruses compared. Each determination was duplicated, the results of the 2 separate trials being listed together in table 4 for convenience of comparison.

Thermal inactivation points between 60° and 65° C. were found for VCM, CM, and TV1, the 3 viruses that induce color removing or bleaching breaks in tulip, while points between 55° and 60° C. were found for LT and TV2, the viruses that induce color-adding, or intensifying, breaks in tulips. These data are consistent, and also in good agreement with the previous data for CM (Table 3); but McWhorter (13), who determined thermal inactivation points for TV1 and TV2 by inoculation from tulip to tulip, reports end points between 65° and 70° C. for both viruses with the comment "we have been unable to distinguish or separate the 2 viruses on a physical property basis."

The dilution end points found are less consistent than the thermal inactivation points. The differences between duplicate determinations on a single virus are as large as those between different viruses in a given test, except that LT yielded uniformly low end points. Dilution is the best index of concentration available for these viruses, and variable results such as these may indicate wide differences in concentration between samples assumed to be comparable. Such differences may be expected to affect other property determinations. According to these data the dilution end point of TV1 is beyond 10^{-4}, that of VCM between 10^{-4} and 10^{-5}, that of CM between $10^{-3.5}$ and 10^{-4}, that of TV2 between 10^{-3} and $10^{-3.5}$, and that of LT between 10^{-1} and 10^{-2}. Again it should be noted that McWhorter (13) found no differ-

TABLE 3.—*Properties of the Easter lily mottle virus (CM)*

Source of virus	Test species	Thermal inactivation point: Temperature, °C. during 10 min. exposure							
		Unheated	50°	55°	60°	65°	70°	75°	80°
L. formosanum	*L. formosanum*	10/10[a]	9/10	10/10	7/10	0/10	0/10	0/10	0/10
" "	" "	10/10	10/10	10/10	0/10	0/10	0/10	0/10	.....
" "	" "	10/10	10/10	10/10	9/10	0/10	0/10	0/10	.....
Easter lily sdlg.	Clara Butt tulip	13/15	10/15	5/14	0/13	0/15	0/15	.....	.....
Norwood Easter lily	" " "	4/15	1/15	0/15	0/15	0/15	0/15	.....	.....

Source of virus	Test species	Dilution end point: Ratio of dilution of extracted sap								
		1/10	1/30	1/100	1/300	1/1,000	1/3,000	1/10,000	1/100,000	1/1,000,000
L. formosanum	*L. formosanum*	10/10[a]	.....	10/10	.....	10/10	.....	0/10	0/10	0/10
" "	" "	10/10	.....	10/10	.....	9/10	.....	0/10	0/10	0/10
Easter lily sdlg.	Clara Butt tulip	6/15	0/15	2/13	0/13	0/15	0/14	0/14	.....	.....
Norwood Easter lily	" " "	1/14	0/15	0/15	0/15	0/14	0/15	0/15	.....	.....

Source of virus	Test species	Tolerance of aging: Number of days extracted sap was aged at 18° C.							
		0	1	2	3	4	5	6	10
L. formosanum	*L. formosanum*	.....	9/10	0/10	0/10	0/10	.....	0/10	.....
Easter lily sdlg.	Clara Butt tulip	10/14[a]	1/15	0/15	0/15	.....	0/15	.....	0/14
Norwood Easter lily	" " "	0/15	0/15	0/14	0/13	.....	0/14	.....	0/14

Source of virus	Test species	Tolerance of drying: Number of days dried on cloth							
		0	1	2	3	5	10		
Easter lily sdlg.	Clara Butt tulip	13/15[a]	0/15	0/15	0/15	0/15	0/15	.....	.....
Norwood Easter lily	" " "	0/14	0/9	0/14	0/10	0/15	0/15	.....	.....

[a] Number of plants infected over number exposed.

TABLE 4.—*Properties of 5 viruses of the tulip group, as determined in sap of Lilium formosanum tested on L. formosanum*

Virus	Test No.	Thermal inactivation point					
		Temperature, °C. during 10 min. exposure					
		Unheated	50°	55°	60°	65°	70°
VCM	3	5[a]	5	5	2	0	0
	8	5[a]	4	5	4	0	0
CM	3	5	5	5	5	0	0
	8	5	5	5	5	0	0
LT	3	2	5	1	0	0	0
	8	5	4	1	0	0	0
TV1	3	5	5	5	5	0	0
	8	4	5	2	0	0	0
TV2	3	4	5	4	0	0	0
	8	4	4	4	0	0	0

		Dilution end point					
		Ratio of dilution of extracted sap					
		1/10	1/100	1/1,000	1/3,000	1/10,000	1/100,000
VCM	4	7[b]	6	2	0	0	.....
	7	8[b]	8	4	3	1	0
CM	4	8	7	1	2	0	.....
	7	8	8	4	3	0	0
LT	4	7	0	0	0	0	0
	7	7	0	0	0	0	0
TV1	4	8	8	3	3	3	.....
	7	8	6	4	0	0	0
TV2	4	5	1	1	0	0	.....
	7	8	5	0	0	0	0

		Tolerance of aging						
		Number of days extracted sap was aged at 18° C.						
		0	1	2	3	4	5	6
VCM	2	10[c]	9	9	.....	.....	2	.....
	5	5[a]	5	5	4	5	4	3
CM	2	10	10	10	.....	.....	1	.....
	5	5	5	4	3	5	4	0
LT	2	9	6	3	.....	.....	0	.....
	5	5	5	3	1	3	0	0
TV1	2	10	9	8	.....	.....	0	.....
	5	5	5	5	4	4	5	4
TV2	2	10	0	0	.....	.....	0	.....
	5	5	5	1	2	3	0	0

		Tolerance of drying						
		Number of hours dried on cloth						
		0	2	4	6	8	24	32
VCM	1	10[c]	.....	0	.....	0	0	0
	6	5[a]	3	1	2	.....	.....	.....
CM	1	10	.....	0	.....	0	0	0
	6	5	2	2	0	0	0	0
LT	1	8	.....	0	.....	0	0	0
	6	3	0	0	0	.....	.....	.....
TV1	1	10	.....	0	.....	0	0	0
	6	5	4	2	0	.....	.....	.....
TV2	1	8	.....	0	.....	0	0	0
	6	5	0	0	0	.....	.....	.....

[a] Number of plants infected of 5 exposed.
[b] Number of plants infected of 8 exposed.
[c] Number of plants infected of 10 exposed.

ences in dilution properties between TV1 and TV2, reporting both infectious at dilutions of 10^{-5}.

The tests on tolerance of aging were not continued beyond 6 days, as earlier determinations for CM (Table 3) indicated an end point between 1 and 2 days. These tests failed to establish end points for VCM and TV1, both of which were active after 6 days' aging. The CM virus was active after 5 days but not after 6 days, and both LT and TV2 produced infection after 4 days' but not after 5 days' aging. Guterman (6) found the virus causing mottling of *Lilium speciosum* Thunb. var. *rubrum* Masters to be infectious to this species after aging 2 hours but not after $2\frac{1}{2}$ hours or more.

In the first experiment on tolerance to drying on cloth squares none of the 5 viruses were active after 4 hours or more, but in a duplicate experiment VCM was found infectious after 6 hours' drying, CM and TV1 after 4 hours', but not after 6 hours' drying, while TV1 and TV2 failed to withstand 2 hours' drying under these conditions. McWhorter (13) reports

TABLE 5.—*End points determined for 4 physical properties of mottle viruses*

Virus	Thermal inactivation (°C.)	Dilution	Aging (days)	Drying (hours)
VCM	60	10^{-4}	6+	6
TV1	60	10^{-4}	6+	4
CM	60	$10^{-3.5}$	5	4
TV2	55	10^{-3}	4	0
LT	55	10^{-1}	4	0

TV1 and TV2 still active after 11 days' drying in tulip leaves. Guterman (6) found no virus capable of infecting *Lilium speciosum rubrum* in leaves of this species or leaves of *L. auratum* or *L. longiflorum* that had dried completely between blotters.

The experimentally determined end points for the 5 viruses are collected for convenient reference in table 5. Although the differences between extremes may be considered sufficiently great to separate distinct viruses, the 5 viruses obviously form a related series. The 3 viruses VCM, TV1, and CM, which are the ones causing color-removing breaks in tulips, show close agreement in properties, with tolerances higher than those of the 2 color-adding viruses TV2 and LT.

VECTOR RELATIONS OF THE MOTTLE VIRUSES

Only *Aphis gossypii* had been established as a vector of lily viruses when the present study was undertaken. Ogilvie (17) found this species to be the sole vector of lily-rosette virus. Guterman (6) found that *A. gossypii* transmitted a virus from mosaic *Lilium speciosum rubrum* to *L. speciosum rubrum* (10/10, 8/10), to *L. longiflorum formosum* (6/10, 13/20), and to *L. auratum* (4/5); from *L. auratum* to *L. longiflorum formosum* (3/5) and to *L. speciosum rubrum* (6/8); and from *L. longiflorum formosum* to *L.*

speciosum rubrum (4/5). He also showed each of 4 stages of this vector to be capable of transmitting the virus, and proved a single aphid could transmit it. In similar trials Guterman obtained negative results with *A. ogilvei* Theob., *A. fabae* Scop., *Macrosiphum solanifolii* Ashm., *Myzus circumflexus* Buckt., and *M. persicae*. Guterman's results cannot be satisfactorily correlated with the writers' at present, because he dealt largely with the virus causing stripe mottling in *L. speciosum* and *L. auratum*, species not yet studied in detail by the present writers.

The demonstration (2, 12, 14) that certain lily viruses could induce breaks in tulips closely similar to those brought about by the tulip-breaking viruses in nature suggested that these lily viruses might be transmissible by the vectors of tulip-breaking viruses, *Macrosiphum solanifolii* and *Myzus persicae* (4, 9, 10, 11). A study of vectors of lily viruses, with particular emphasis on Easter lily viruses, was therefore undertaken in 1939.

Methods

Plants were moved to a separate insectary greenhouse for aphid inoculation trials. In the early experiments non-viruliferous aphids were colonized on infected source plants and allowed to feed for several days or longer before they were transferred to healthy test plants. In later tests such non-viruliferous aphids were transferred from favorable food plants to Petri dishes containing pieces of leaves, stems, or flowers from the diseased source plant and allowed to feed for 1 to 3 hours. When such source material was held at about 18° C. the aphids settled down and fed readily but wandered about and congregated on the top or sides of the dish when temperatures were higher. These pieces of source plant material with the aphids in place were then transferred by forceps to pieces of paper resting on leaves of the healthy plants being inoculated, thus avoiding contact of the diseased material with the healthy plants. Under these conditions the aphids moved freely to the leaves of the test plants as the source material withered. After 1 to 5 days' feeding the survivors were destroyed by pyrethrum or rotenone sprays, and the plants were returned to the aphid-free greenhouse for observation.

Aphis gossypii extricates its mouth parts from the plant tissue with greater difficulty than do the other species investigated and was therefore transferred by brush less readily. It may be that lower percentages of disease transmission by this species were due in part to injury of individuals during handling. This did not apply to *Macrosiphum solanifolii*, however, which is readily transferred by brush.

Experimental Results

The data in table 6 show that *Macrosiphum solanifolii* and *Myzus persicae*, as well as *Aphis gossypii* are vectors of CM. No transmission was effected by *A. fabae*, *Macrosiphum lilii* Monell, *Myzus circumflexus*, or *Myzus convolvuli* Kalt. under comparable conditions. Negative results were

confirmed by mechanical subinoculation to *Lilium formosanum*. It is of interest that *Macrosiphum lilii, Myzus convolvuli,* and *Myzus circumflexus* colonize readily on Easter lilies but do not transmit this virus, while *Aphis gossypii* is the only vector species with similar host preferences. *Macrosiphum solanifolii* and *Myzus persicae* will colonize maturing healthy plants but die within a few days on younger growth.

Aphis gossypii proved to be comparatively inefficient as a vector of CM. Its failure to transmit from Easter lily to *Lilium tigrinum* is particularly surprising inasmuch as this transfer is easily accomplished mechanically. In view of this failure little weight is placed on the negative results on *L. humboldtii magnificum* and *L. parvum*. *Macrosiphum solanifolii* was also inefficient in these tests, and even *Myzus persicae* gave low proportions of transfer in earlier trials; but in later tests, with use of the Petri-dish method and short feeding periods on source plant tissue, it was highly efficient. This species was utilized in comparative trials with VCM, TV1, TV2, and LT. If an incubation period of these viruses occurs in *Myzus persicae* it is less than 1 day's duration since successful transfers were obtained within that period.

VCM was readily transmitted by *Myzus persicae* (Table 7) from Easter lily to Easter lily, *Lilium tigrinum,* and tulip, and somewhat less readily to *Ornithogalum thyrsoides*. When established in *Ornithogalum,* this virus was readily transferred to *Ornithogalum,* Easter lily, and tulip. Symptoms induced in *L. tigrinum* on transfer of VCM by *M. persicae* agreed fully with those produced mechanically, including initial gray surface flecking with subsequent progressive yellowing and death. As already noted, *M. persicae* in parallel trials failed to transmit VCM to onion, *Allium cernuum, A. fistulosum, A. porrum,* or *Iris filifolia*. *Myzus circumflexus* from similar source material failed to transmit VCM to tulip, Easter lily, or *Ornithogalum;* no evidence has been found that this species can transmit either CM or VCM.

In parallel trials (Table 7) *Myzus persicae* transferred TV1, TV2, and LT to tulip with high efficiency, but failed to transfer any of these to *Ornithogalum thyrsoides*. Symptoms induced in tulips on transfer of the viruses by *M. persicae* agreed closely with those produced on mechanical inoculation with the same viruses. Symptoms tended to appear earlier following aphid transfer. Clara Butt tulips, exposed to aphids soon after the leaves appeared above the soil, developed recognizable leaf symptoms 11 days after introduction of TV1 in one test. Other viruses producing strong leaf symptoms (CM, VCM) sometimes produced symptoms in 15 or 16 days, but TV2 and LT, which mottle tulip leaves weakly or not at all, were not positively diagnosed until the tulips flowered, 30 to 38 days or longer after inoculation.

In a few additional trials (Table 8) commercial Rembrandt tulips, carrying TV1 and TV2 in mixture according to McWhorter's (15) interpretation, served as source plants and *Lilium formosanum* as test plants. The estab-

TABLE 6.—*Transmission of lily mottle virus (CM) by aphids*

Vector species	Source of virus	Plants exposed	No. tests	Plants affected[a]	Minimum incubation period (days)	Symptoms
Aphis fabae	*L. formosanum*	*L. formosanum*	1	0/2	.....	None
A. gossypii	Easter lily	Easter lily sdlgs.	18	5/193	10	Mottle
" "	" "	*L. formosanum*	6	9/26	13	"
" "	" "	Clara Butt tulip	5	10/28	36	Flower break
" "	" "	*L. tigrinum*	2	0/15	.....	None
" "	" "	*L. humboldtii magnificum*	1	0/15	.....	"
" "	" "	*L. parvum*	1	0/5	.....	"
" "	*L. formosanum*	Easter lily sdlgs.	1	0/15	.....	"
" "	*L. candidum*	" " "	2	9/20	29	Mottle
" "	*L. formosanum*	*L. formosanum*	18	7/88	17	"
Macrosiphum lilii	Easter lily	" "	3	0/20	.....	None
" "	*L. formosanum*	" "	7	0/37	.....	"
M. solanifolii	Easter lily	Easter lily sdlgs.	1	1/2	20	Mottle
" "	" "	Clara Butt tulip	2	1/20	31	Flower break
Myzus circumflexus	" "	Easter lily sdlgs.	4	0/40	.....	None
M. convolvuli	" "	" " "	4	0/11	.....	"
M. persicae	" "	Clara Butt tulip	4	29/68	15	Flower break
" "	" "	Easter lily sdlgs.	6	12/39	16	Mottle
" "	" "	*Ornithogalum thyrsoides*	1	0/5	.....	None
" "	*L. formosanum*	Clara Butt tulip	1	5/5	15	Flower break

[a] Number of plants infected over number exposed.

lished vectors of tulip breaking (4, 9, 10, 11), *Macrosiphum solanifolii* and *Myzus persicae*, effected transfer of viruses as expected, and 2 species that failed to carry lily mottle viruses, *Macrosiphum lilii* and *Myzus circumflexus*, also failed to carry tulip-breaking viruses. *Aphis gossypii*, an inef-

TABLE 7.—*Transmission of the mottle viruses VCM, TV1, TV2, and LT by Myzus persicae*

Source of virus	Plants exposed	No. tests	Plants affected[a]	Minimum incubation period (days)	Symptoms
Virulent mottle virus (VCM)					
Easter lily	Easter lily sdlg.	15	67/93	11	Mottle, leaf distortion
" "	*L. tigrinum*	1	5/5	8	Gray flecks, yellowing, death
" "	Clara Butt tulip	4	29/35	16	Mottle, flower-break
" "	*Ornithogalum thyrsoides*	4	9/20	22	Mottle
Ornithogalum thyrsoides	"	1	4/5	26	"
"	Easter lily sdlg.	1	4/5	14	Mottle, leaf distortion
"	Clara Butt tulip	1	5/5	15	Mottle, flower-break
L. formosanum	" " "	1	5/5	15	" "
Tulip virus 1 (TV1)					
L. formosanum	Clara Butt tulip	2	8/8	11	Mottle, flower-break
" "	*Ornithogalum thyrsoides*	1	0/5	.....	None
Tulip virus 2 (TV2)					
L. formosanum	Clara Butt tulip	1	5/5	15	Mottle, flower-break
Clara Butt tulip	" " "	1	4/4	30	" "
L. formosanum	*Ornithogalum thyrsoides*	1	0/5	.....	None
Latent virus (LT)					
L. formosanum	Clara Butt tulip	1	5/5	38	Mottle, flower-break
" "	*Ornithogalum thyrsoides*	1	0/5	.....	None

[a] Number of plants infected over number exposed.

ficient vector of lily-mottle viruses, effected a single transfer of tulip-breaking virus. *Aphis fabae*, little tested by workers on either tulips or lilies, proved very efficient in transmitting tulip-breaking viruses. The last 2 species, *A. fabae* and *A. gossypii* are here recorded for the first time as vectors of tulip-breaking viruses.

TABLE 8.—*Transmission of virus from commercial Rembrandt tulips to Lilium formosanum by aphids*

Vector species	Source of virus	Plants exposed	No. tests	Plants affected[a]	Minimum incubation period (days)	Symptoms
Aphis fabae	Rembrandt tulip	*L. formosanum*	2	8/9	13	Mottle
A. gossypii	"	"	1	1/8	19	"
Macrosiphum lilii	"	"	1	0/8	.....	None
M. solanifolii	"	"	1	4/8	19	Mottle
Myzus circumflexus	"	"	1	0/8	.....	None
M. persicae	"	"	1	6/8	19	Mottle

[a] Number of plants affected over number exposed.

TABLE 9.—*Protective effects of some lily mottle viruses against other viruses of this group*

Mottle virus first introduced	Date of first inoculation	Method of determining presence of first virus	Mottle virus introduced second	Date of second inoculation	Symptoms expressed	Protective effect
CM (?) (masked) in Croft Easter lily	Natural infection	Indexing	CM	(1938) Dec. 8	Trace of mottling	Essentially complete
CM (masked) in Florida lily	"	"	CM	Dec. 8	None	Complete
CM (masked) in Norwood Easter lily	"	"	CM	(1940) Feb. 10	"	"
"	"	"	VCM	(1943) Feb. 17	"	"
"	"	"	VCM[a]	Mar. 9	"	"
CM in Easter lily sdlg.	(1943) Feb. 17	Symptoms	VCM	Mar. 24	"	"
LT (masked) in Easter lily sdlg.	Feb. 17	Indexing	VCM	Mar. 24	Mottling, distortion	None
LT in *L. tigrinum*	(1942) June 4	"	CM	Mar. 24	Etch, yellowing, death	"
TV1 (masked) in Easter lily sdlg.	(1943) Mar. 24	"	VCM	Apr. 26	Mottling, distortion	"
TV2 (masked) in Easter lily sdlg.	Mar. 24	"	VCM	Apr. 26	"	"

[a] Second virus added by *Myzus persicae* here, in all other tests by mechanical methods.

PROTECTIVE ACTION OF CERTAIN MOTTLE VIRUSES AGAINST CM AND VCM

Inasmuch as CM and VCM produce symptoms in Easter lilies and TV1, TV2, and LT do not, the Easter lily may serve as a test plant for protective action of certain of these viruses against CM or against VCM. Also *Lilium tigrinum*, which is mildly affected by LT, TV1, or TV2, but killed by CM or VCM, has similar advantages for protective tests. Tests of this type that have been completed are summarized in table 9.

The CM symptoms could not be induced in certain commercial Easter lily stocks (Croft, Florida, Norwood Creole) naturally infected with this

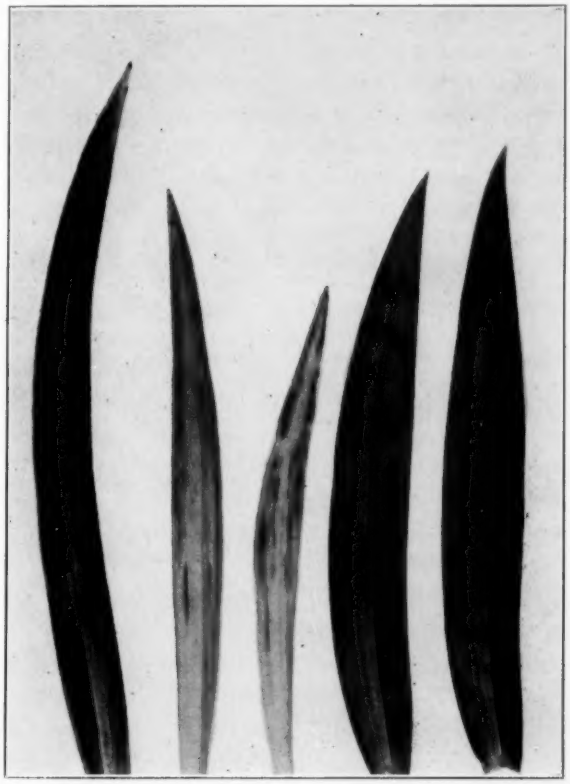

FIG. 11. VCM superimposed on TV1 in 3 leaves at left with, right, 2 leaves TV1 controls. TV1 introduced Mar. 24, 1943; presence established by indexing. VCM introduced April 26, 1943. Photographed June 9, 1943, by Mead. VCM was similarly superimposed on TV2 and LT in separate trials but not on CM.

or a similar virus but showing no symptoms. Similarly VCM failed to induce symptoms in Norwood Creoles when introduced mechanically or by *Myzus persicae*, and VCM failed to develop further distinctive symptoms when superimposed on CM experimentally produced and expressing the usual mottling symptoms in Easter lily seedlings. In contrast VCM showed full expression of symptoms when superimposed on LT, TV1 (Fig. 11), or TV2, previously established but symptomless in Easter lily seedlings. In a

single test in *Lilium tigrinum*, LT established in the previous season of growth showed no protective action against CM when this virus was superimposed (Fig. 10).

The data thus far available on protective action suggest that CM and VCM are closely allied, but distinguish these from TV1, TV2, and LT. This separation is in agreement with the test reactions in Easter lily and in *Lilium tigrinum* (Table 1) but at variance with the test reactions in tulip and with the property values (Table 5) which place TV1 in the group with CM and VCM.

NEGATIVE EVIDENCE ON SEED TRANSMISSION OF LILY VIRUSES

Inasmuch as masking of CM is so common in Easter lilies as to make roguing impracticable, seedlings appear to offer the only means of producing virus-free stocks. LT, and probably other viruses of the mottle group, presents similar problems in various garden species. Evidence that these viruses are not seed-borne is therefore basic to any program of producing virus-free lilies.

TABLE 10.—*Number of seedlings grown from mosaic Easter lily plants and showing no evidence of seed-borne virus*

Symptoms in ♀ parent	Symptoms in ♂ parent				
	Mottling	Necrotic fleck	None	Unknown	Totals
Mottling	923 (23)[a]	588 (9)	2748 (56)	207 (8)	4466 (96)
Necrotic fleck	743 (16)	233 (34)	1867 (34)	69 (3)	2912 (57)
None	602 (18)	870 (16)	1326 (29)	2736 (53)	5534 (116)
Totals	2268 (57)	1691 (29)	5941 (119)	3012 (64)	12912 (269)

[a] Total number of seedlings, with number of progenies in parentheses.

Guterman's (6) excellent data on seed transmission, which have not heretofore been made available to most workers, are worth presenting here. He detected no mosaic symptoms in the following numbers of seedlings from mosaic parent species: *Lilium candidum* 91, *L. davuricum* 878, *L. henryi* 74, *L. longiflorum* 84, *L. regale* 487, *L. sargentiae* 270, *L. speciosum rubrum* 252, *L. tenuifolium* 253, *L. tenuifolium* var. Golden Gleam 89, *L. umbellatum* 139. He concluded that the virus of lily mosaic is not transmitted in the seed.

The writers have data (Table 10) on seedlings of *Lilium longiflorum* only. In connection with breeding research on this species, seeds of 269 progenies were sown in greenhouses in September, 1936, grown continuously under glass to the 3-inch-pot stage, and inspected for virus symptoms until they were dried off in July, 1937. The virus symptoms expressed in each parent plant were recorded except for open-pollinated progenies of which the male parents were unknown. Extensive indexing in tulip and *L. formosanum* indicates that practically all commercial Easter lilies carry a mottle virus even if symptomless, and that necrotic fleck in commercial

Easter lilies is uniformly accompanied by a virus of this group. Accordingly, all of the 12,912 seedlings (Table 10) produced from commercial Easter lily parents would appear to contribute valid evidence against seed carriage of viruses of the mottle group in this species. The possibility of masking in these test seedlings themselves has been considered, but indexing of numbers of random samples of these on *L. formosanum* and tobacco has yielded no evidence that either mottle viruses or cucumber-mosaic virus can be seed-borne but masked. It may therefore be fairly stated that no evidence is known that any lily virus is seed-borne.

DISCUSSION AND CONCLUSIONS

Although the lily viruses VCM, CM, and LT are those of chief interest in the present study, the tulip viruses TV1 and TV2 are intimately concerned in the problems of classification and nomenclature. Smith (19) included TV1 and TV2 under the name Tulipa Virus 1 (Cayley), and, accepting Atanasoff's (1) account, listed hyacinth mosaic and narcissus mosaic as caused by the same virus. Holmes (8) erected the species *Marmor tulipae* for TV2, and *Marmor mite* for TV1 and Lily-latent virus, combining the two last named on the strength of the limited evidence published by McWhorter (14) up to that time. It is now apparent that the symptoms McWhorter assigned to his latent virus in tulips resembling those of TV1, although characteristic of viruses from the source species *Lilium candidum* and *L. longiflorum*, do not fit our LT from *L. tigrinum* which has much more in common with TV2. In earlier accounts of the "strong mottle of Easter lily" Brierley (2, 3) did not attempt to class this virus farther than "the tulip group" of viruses, namely the group studied herein. The virulent mottle virus VCM is reported for the first time in the present paper.

It is evident that the data presented above would serve to set up and name 2, 3, 4, or even 5 separate virus entities, without violating commonly accepted standards for differentiating mosaic viruses. Thus symptoms in Easter lily and *Lilium tigrinum* (Table 1) and protection reactions in these 2 species, place the 5 cultures in 2 groups: VCM–CM and TV1–TV2–LT. But the tulip reactions, and the properties as determined in *L. formosanum* (Table 5), serve to group them differently: VCM–CM–TV1 and TV2–LT, while the reaction of *Ornithogalum* tends to set VCM apart as a distinct entity. Thus VCM and CM form a natural group, with one host reaction difference (in *Ornithogalum*) and symptom differences in Easter lily that are of the order expected of strains. Similarly TV2 and LT appear closely allied, with strain differences in symptom expression in *L. formosanum* and *L. tigrinum*. It is TV1 that fails to conform to either of these groups, showing affinity to the first in tulip reaction and in properties, but resembling the second group in Easter lily reaction and in protection relations.

However, the writers are reluctant to add further to the confusion in nomenclature, and prefer to use a single binomial for the group, indicating the 5 members studied as sub-species or strains. This requires amending

Marmor tulipae Holmes (8) to include color-removing, as well as color-adding, break symptoms in tulips. Tulip-breaking virus, so designated, has a host range in Liliaceae of *Lilium formosanum, L. longiflorum, L. tigrinum,* and *Tulipa gesneriana,* with many other species of lilies presumably susceptible, and a few other hosts known for specific strains. Symptoms vary with the strains, as shown in table 1. Transmission by sap and by *Aphis gossypii, Macrosiphum solanifolii,* and *Myzus persicae,* but not through seed, characterizes *Marmor tulipae* thus defined. *Anuraphis tulipae* Boyer (11), and *Aphis fabae* (herein) are recorded as vectors of tulip color-adding and tulip color-removing strains. Properties as determined in *L. formosanum* also vary with the strain (Table 5) : thermal inactivation point 60° to 65° C. or lower; dilution end-point 10^{-4}, or at less dilution; active in expressed juice after 6 days (4 days), and after drying on cloth for 6 hours (0 hours). This conception of *Marmor tulipae* is in agreement with Tulipa virus 1 (Cayley) in Smith (19) except that the reported hosts *Hyacinthus* and *Narcissus* must be omitted.

The following sub-species and strains of tulip-breaking virus may be described from the data provided in this paper:

Tulip-color-removing sub-species (the type sub-species). Predominantly color-removing breaks in tulip flowers; symptomless in Easter lily; green mottling soon masked in *Lilium tigrinum;* sparse and fine green island mottling in *L. formosanum;* thermal inactivation point 60° to 65° C.; dilution 10^{-4}; withstands aging 4 days, drying 4 hours; fails to protect against virulent mottle virus (VCM) in Easter lily.

Synonyms: Tulip color removing strain McWhorter (15) part of *Marmor mite* Holmes (8).

Tulip-color-adding sub-species. Predominantly color-adding breaks in tulip flowers; symptomless in Easter lily; non-fatal effects in *Lilium tigrinum;* mottling without yellowing or dwarfing in *L. formosanum;* thermal inactivation point 55° to 60° C.; dilution 10^{-1} to 10^{-3}; withstands aging 4 days; fails to withstand drying 2 hours; fails to protect against virulent mottle virus (VCM) in Easter lily.

Strains: TV2, tulip-color-adding strain McWhorter (15), *Marmor tulipae* (8), coarse dark and light green mottling in *Lilium formosanum,* gray surface etch in *L. tigrinum;* LT (of this paper), part of lily latent virus McWhorter (14), part of *Marmor mite* Holmes (8), green island mottling in *L. formosanum,* green mottling soon masked in *L. tigrinum.*

Lily-mottle sub-species. Predominantly color-removing breaks in tulip flowers; mottling in Easter lily; gray surface etch followed by general yellowing and death in *Lilium tigrinum;* mottling, yellowing, and dwarfing in *L. formosanum.* Properties in *L. formosanum:* thermal inactivation point 60° to 65° C.; dilution $10^{-3.5}$ to 10^{-4}; withstands aging 5 days or more, drying 4 to 6 hours.

Strains: CM (coarse mottle), strong mottle of Easter lily (3), mottling without distortion in Easter lily; VCM (virulent mottle), leaf and flower distortions in Easter lily, mottling in *Ornithogalum thyrsoides.*

The rank assigned these sub-species and strains may be questioned by others who attach relatively greater importance to certain of the criteria employed. The writers consider host and vector relations of fundamental importance, and find the 5 cultures studied to be relatively uniform in these respects. No great weight is attached to minor differences in host range and properties, for we have found similar differences among recognized strains of cucumber-mosaic virus. Protection tests of the type reported here, namely attempts to superimpose a second systemic infection on a similar first infection, are not in themselves considered decisive as tests of relationship.

The rank properly assigned these cultures is also dependent on their inherent constancy. Although little direct evidence of inconstancy can be cited, there is some reason to believe that these types are too variable to be separated as valid species. VCM is assigned strain rank as a mutant of CM, but no other evidence of mutation of one type to another is available. However, the position of TV1 as a connecting link between the other two sub-species described here suggests definite continuity in the group as a whole. Also, TV1 and TV2, the types on which McWhorter (15) and Holmes (8) have erected separate species, occur in mixture in nature, and have been separated (15) only by sub-culturing from natural segregates. Of these, TV1 is difficult to maintain in tulip, tending to kill the affected plants (15). But neither TV1 nor TV2 is lethal to *Lilium formosanum*, and our data on properties (Table 4) suggest that TV1 may be separable from TV2 by its greater tolerance to aging or drying. Unless some such technique can be shown to separate entities producing distinctive break symptoms in tulips, it does not seem advisable to accord TV1 and TV2 more than sub-specific rank, if they indeed merit more than strain designation.

Lack of seed transmission and the limited host range of lily-mottle strains (VCM, CM, LT) show that spread of these viruses in nature must take place almost exclusively from lily to lily. *Calochortus, Fritillaria,* and *Zygadenus,* though susceptible to CM, like *Lilium* spp. from the wild, appear to be free from virus unless they have been contaminated from diseased lilies. Tulips are often affected with tulip-breaking virus in nature, and as these plants mature when many lilies are in active growth, some spread from tulips to lilies may be expected. The fact that the virus strains thus far detected in lilies are usually different from the tulip strains indicates, however, that such spread is uncommon. Lily seedlings adequately isolated from bulb-propagated lilies should remain virus free. Easter lily seedlings well isolated at Willard, North Carolina (1 season), and at Charleston, South Carolina (3 seasons), did remain virus free, as determined by symptoms and by indexing samples; but similar seedlings inadequately isolated elsewhere developed both mottle and necrotic fleck. Guterman (6) reached similar conclusions, his inoculations to and from lily revealing only one host, a *Fritillaria,* outside the genus *Lilium*. He adds that "a large number of cases have been under observation for periods of 3 and 4 years in which

healthy groups of lilies growing in gardens in various localities far removed from any mosaic plants of the same genus have remained healthy during this time even though the known insect vector of the virus was repeatedly observed feeding upon them."

Although production of virus-free lilies from seed appears feasible, Easter lily seedlings are too variable for forcing use unless selected for this purpose. American growers seem unanimous in the conclusion that production of virus-free Easter lilies is not commercially practicable. Easter lilies force satisfactorily when affected with CM, but direct comparisons of the performance of virus-free and CM stocks of the same clon have not been made. This virus strain, or allied types, is apparently universal in commercial Easter lily stocks, but is so frequently masked that roguing is not feasible. The soundness of the decision to ignore mottle in commercial Easter lily production was thrown into question once more with the appearance of virulent mottle (VCM), which produces some cull plants. It became of immediate importance to learn whether VCM is a mutant from CM or a distinct virus. The evidence detailed above is believed adequate to show that it is a mutant of CM. Physical properties of the two agree closely (Table 5), no differences in vector relations are known, and but one additional host, *Ornithogalum thyrsoides*, is known for VCM. Protection tests (Table 9) indicate very close relationship, for VCM was not superimposed on CM by mechanical methods or by *Myzus persicae*. This last finding indicates that VCM cannot spread into Creole lilies, but that it arises spontaneously from the CM virus already present in these, for VCM is known to appear in Creole lilies under circumstances that simulate natural spread. The VCM type has been found to appear naturally only in stocks of CM history. In some transfers by *Myzus persicae* from Easter lilies of supposedly pure CM content to Easter lily seedlings, the VCM symptoms have appeared, suggesting that this vector picked up such mutant types not recognizable in the source plant by symptom expression.

If CM is to be ignored in commercial Easter lily production because it cannot be effectively rogued, the same logic requires that VCM be ignored also. If VCM arises spontaneously from CM, as our evidence indicates, roguing the plants that express VCM symptoms will not eliminate this virus. In our experience the incidence of VCM in forced Creole lilies is comparatively low, and, in affected plants the virulent symptoms are not always expressed at the top where they would result in cull blooms, so that the proportion of unsalables has not been high. As the recurrence of the virulent symptoms from year to year also appears to be low, VCM does not appear to be a serious menace in field production.

More or less seasonal waves of migrant aphids are evidently responsible in large part for the general prevalence of mottle viruses in lilies. Potatoes (*Solanum tuberosum* L.) and cruciferous crops (*Brassica* spp., etc.) are favored food plants of *Myzus persicae*. When such crops mature, or when they become heavily infested, the aphids develop winged migrants in

large numbers. These migrants carry no virus from potatoes or crucifers to lilies, and do not prefer lilies as food plants. When they encounter lilies, however, they do feed briefly on a great many individual plants, and thus prove highly efficient vectors of lily mottle viruses that are already present. Spread of lily-mottle virus by *M. persicae* under such circumstances is clearly recognizable in indicator species such as *Lilium formosanum.*

SUMMARY

Three mottle viruses from lilies, a latent type from *Lilium tigrinum* (LT), the strong mottle of Easter lily (CM), and a more virulent mutant from the latter (VCM), are compared with McWhorter's Tulip Viruses 1 and 2 in the test species Easter lily, *L. formosanum, L. tigrinum,* and tulip.

The host range of CM is found limited to Liliaceae, including *Calochortus* sp., *Fritillaria pudica,* tulips, and *Zygadenus fremontii,* in addition to several species of lilies. The known ranges of the other 4 viruses are similar except that the VCM strain infects also *Ornithogalum thyrsoides.*

Properties of the 5 viruses were determined in *Lilium formosanum.* The thermal inactivation points of VCM, CM, and TV1 were found to lie between 60° and 65° C.; those of TV2 and LT, between 55° and 60° C.

Two viruses, VCM and TV1, were active after dilution to 10^{-4}, CM at $10^{-3.5}$, TV2 at 10^{-3}, and LT at 10^{-1}. All 5 viruses were active after aging 4 days at 18° C., CM after 5 days, and VCM and TV1 after 6 days. VCM survived 6 hours' drying on cloth, CM and TV1 survived 4 hours, while TV2 and LT were inactive after drying 2 hours.

CM is transmitted by the aphids *Aphis gossypii, Macrosiphum solanifolii,* and *Myzus persicae,* but not by the common lily infesting species *Myzus circumflexus, M. convolvuli,* or *Macrosiphum lilii. Myzus persicae* carries all 5 viruses with high efficiency. *Aphis fabae* and *A. gossypii,* in addition to the previously established vectors *Macrosiphum solanifolii* and *Myzus persicae,* transmitted virus from Rembrandt tulips to *Lilium formosanum.*

CM, naturally or experimentally established in Easter lilies, was found to offer complete protection against further addition of CM or of VCM. The tulip viruses TV1 and TV2, and LT from *Lilium tigrinum,* similarly established in Easter lily, offered no protection against subsequent addition of VCM. LT in *L. tigrinum* failed to protect this species against CM, which produces killing effects in this host species.

Over 12,000 seedling Easter lilies grown from seed of diseased parents showed no evidence of seed carriage of any lily virus.

It is suggested that the 5 viruses studied be considered strains or subspecies of tulip-breaking virus, which *Marmor tulipae* Holmes may be amended to describe, and which will correspond to *Tulipa virus 1* (Cayley) Smith if the alleged hosts *Hyacinthus* and *Narcissus* are omitted.

VCM is considered a mutant from CM that appears in Easter lily stocks

carrying CM. Easter lily producers, already ignoring CM because it is impracticable to rogue in this species, are advised to ignore VCM also, for the same reason, although the latter produces some cull plants.

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BACTERIAL SOFT ROT OF SPINACH

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Bacterial soft rot of spinach is one of the most frequently observed diseases of spinach in transit and on the market. Records of the Fresh Products Inspection Division, Fruit and Vegetable Branch, United States Department of Agriculture, Chicago, Illinois, during 1936–43 show that this disease caused practically all the decay noted in spinach from all shipping regions. In many shipments there was only a trace, but in others the decay ranged as high as 65 per cent in some baskets, with an average of 40 per cent for the car.

Although many writers have assumed that the soft rot of spinach is caused by *Erwinia carotovora* (L. R. Jones) Holland, no critical studies have been made to determine the identity of the causal organism. This paper is the result of studies on the morphological, physiological, and pathological characteristics of the causal organism of the soft rot of spinach.

The first manifestations of the disease are water-soaked areas on the leaves (Fig. 1, A). These are followed by a rapid softening of the tissues which often become light yellow, wet, and mushy. High humidity and high temperature (24 to 30° C.) favor the rapid spread of the disease, which may completely destroy the leaf. If diseased leaves are removed to dry air the decay may be checked and the affected tissues become dry and brittle (Fig. 1, B).

In the present study three isolates from rotted spinach and two isolates from rotted potatoes were used. Earlier physiological and pathological tests had shown that the potato isolates were authentic *E. carotovora.*

MORPHOLOGY AND STAINING REACTIONS

Methods. Morphological characteristics of the isolates were determined from 48-hour-old cultures grown at 24° C. on beef extract nutrient agar adjusted to pH 6.9. For demonstration of form and size, "negative" preparations from smears were made with one per cent nigrosin. Fisher and Conn's (3) flagella stain was used to determine the number and position of flagella. Gram reaction was determined according to Hucker and Conn's (4) modification of Gram's stain. Ziehl-Nielsen's method was employed for acid-fast properties. The Anthony (1) method of capsule staining was employed.

Morphological characteristics. The isolates from spinach and the *E. carotovora* isolates from potato were rods, 0.4 to 0.6 by 3.6 to 4.5 microns, occurring singly, occasionally in chains; no spores; no capsules. The organ-

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isms were motile by 2 to 5 peritrichiate flagella. They were Gram-negative and not acid-fast. They stained readily with carbol fuchsin and crystal violet. In general it may be stated that the morphological characteristics of the cells of the different isolates were not sufficiently distinct to separate them one from another.

CULTURAL CHARACTERISTICS

Potato-dextrose agar (pH 7.0). On slants, growth of all isolates was thin, grayish-white, moist, glistening, butyrous. Medium was not discolored.

Potato. On steamed potato cylinders the isolates formed a smooth, slimy raised, cream-white growth.

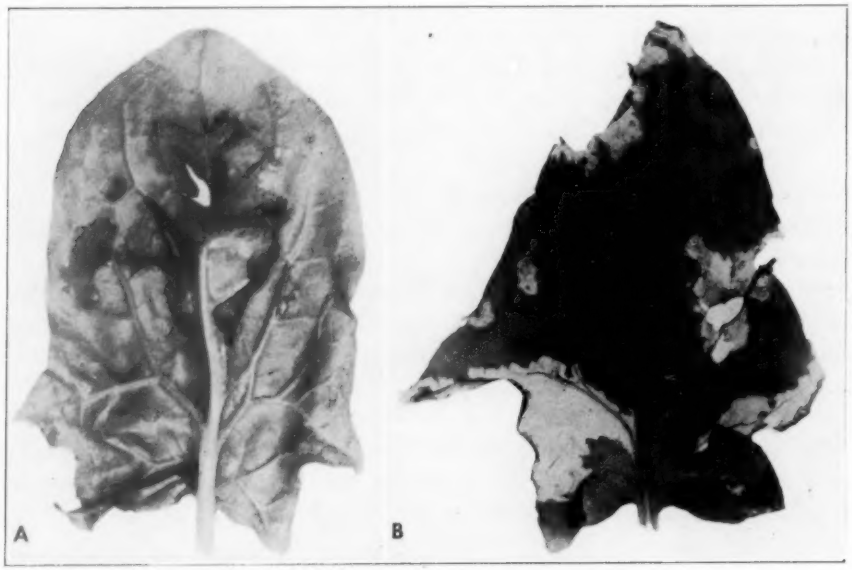

FIG. 1, A. Early stage of bacterial soft rot of spinach showing water soaked areas. B. Diseased areas which have dried and become brittle after exposure to dry air.

Beef-extract-agar streak (pH 6.9). Growth on this medium was grayish-white, glistening, butyrous, thin, margins often becoming more or less irregular.

Beef-extract agar (pH 6.9). In dilution plates after 48 hours colonies were circular, convex, grayish-white, glistening, smooth, with margins entire.

Uschinsky's solution (pH 6.8). Growth of all isolates in this medium was abundant after 24 hours.

Litmus milk (pH 7.0). All isolates produced acid in litmus milk. There was coagulation with separation of whey. No peptonization occurred.

Plain gelatin stabs. Growth became visible after 48 hours. In all cases liquefaction was at first infundibuliform but soon became stratiform. Two of the spinach isolates completely liquefied this medium after 3 weeks. The remaining isolates had completely liquefied the medium after 4 weeks.

PHYSIOLOGY

Certain of the recommendations of the Committee on Bacteriological Technique, Society of American Bacteriologists, as given in the Manual of Methods for Pure Culture Study of Bacteria (5), were closely followed in the biochemical studies. The cultures were made in duplicate and were incubated at room temperature which averaged 24° C.

BIOCHEMICAL CHARACTERISTICS

Relation to free oxygen. When grown in Smith fermentation tubes, growth occurred first in the open arms and then progressed to the domes, indicating that the organisms were facultative anaerobes.

Nitrate reduction. The alpha-naphthylamine-sulfanilic-acid test was employed on 1-, 2-, 5-, 7-, 10-, and 14-day-old cultures of the different isolates. The organism *Escherichia coli* was used as a control. Nitrates were reduced to nitrites in all of the cultures.

Indole production. The isolates in Bacto-tryptophane broth (pH 6.8) were tested by the Kovacs test for indole production at the end of 7, 14, and 21 days. No positive reaction was obtained with any of the isolates. When *Escherichia coli* was used as a control organism, a positive test for indole was obtained.

Ammonia production. Strips of filter paper saturated with a freshly prepared Nessler's solution were hung over 36- and 48-hour beef-extract broth cultures of the various isolates. No ammonia production was observed in any of them.

Hydrogen sulphide. Strips of lead acetate paper failed to turn black when hung over beef extract broth cultures of the various isolates, indicating that no hydrogen sulphide was being formed. *Escherichia coli* used as a control organism gave a positive test for hydrogen sulphide.

Hydrolysis of starch. Streak inoculations were made in beef-extract agar containing 0.2 per cent soluble starch. After five days, the surfaces of the plates were flooded with a saturated solution of iodine in 50 per cent alcohol. None showed any clear zone outside the area of growth, indicating that no diastatic action had occurred.

Hydrogen-ion relations. The spinach and the *E. carotovora* isolates all grew within a pH range of 4.2 to 10.0. Most rapid growth during 48 hours occurred from pH 6.2 to 7.5. Growth in nutrient broth was inhibited at pH 4.0 in the acid range and pH 11.0 in the alkaline range.

The results of the cultural and biochemical tests indicate that the isolates are closely related, if not identical.

CARBON METABOLISM

Sugars, alcohols, and glucosides were added to the modified synthetic medium of Ayres, Rupp and Johnson (2).

The basal medium was adjusted to pH 7.0 and sterilized by autoclaving. Brom-cresol purple at a concentration of 0.02 per cent was added as an

indicator. One per cent of sugar was added to the synthetic base and used as a liquid medium. In order to avoid breaking down during sterilization, the sugars lactose, levulose, maltose, sucrose, and raffinose were sterilized by filtration through a Berkefeld filter. Twenty-four carbon sources were utilized. All media were incubated for 6 days at 27° C. to check their sterility before using. Inoculations were made in duplicate. In all the fermentation studies color change from purple to yellow was considered the index of fermentation.

The three spinach isolates fermented arabinose, rhamnose, xylose, dextrose, levulose, maltose, galactose, mannose, raffinose, sucrose, cellobiose, glycerol, mannitol, sorbitol, dulcitol, inositol, salicin, and arbutin. The *E. carotovora* isolates fermented all of these carbohydrates except maltose, sorbitol, and dulcitol. None of the spinach nor *E. carotovora* isolates fermented melezitose, starch, dextrin, erythritol, inulin, or glycogen.

Gas production. Smith fermentation tubes were employed. One per cent of the various sugars was added to the synthetic base and used as a liquid medium. The sugars lactose, levulose, maltose, sucrose, and raffinose were sterilized by filtration through a Berkefeld filter. The remainder were sterilized by autoclaving. Gas production by the spinach and *E. carotovora* isolates was observed in arabinose, dextrose, xylose, raffinose, sucrose, mannitol, salicin, and arbutin.

The spinach and the *E. carotovora* isolates had similar reactions in the various carbohydrate media used with the exception that the latter did not ferment maltose, sorbitol, and dulcitol. These differences in carbon metabolism are not considered sufficient to differentiate the spinach and *E. carotovora* isolates.

PATHOGENICITY STUDIES

Spinach leaves were washed in tap and distilled water, after which they were divided into two lots. Uninjured leaves in one set of damp chambers were sprayed with water suspensions of the various spinach and *E. carotovora* isolates. A second lot of leaves was wounded by scratching with sterile needles. The leaves were then sprayed with water suspensions of the various isolates, after which they were left at room temperature (25° C.). Scratched and unscratched leaves sprayed with distilled water served as controls. Infection in the form of water-soaked areas around the needle scratches was first apparent 18 hours after inoculation. Numerous, small water-soaked areas were observed on the uninjured, inoculated leaves after 30 hours. All injured and uninjured inoculated leaves were completely rotted after 72 hours. Uninoculated leaves did not become infected. The organisms, readily recovered in pure culture from the infected areas, were used in further inoculation experiments.

Injured and uninjured spinach plants growing in pots in the green-house were sprayed with 24-hour-old suspensions of the various isolates. Injured and uninjured plants sprayed with distilled water served as controls. Water-soaked areas in the immediate vicinity of needle scratches were appar-

ent on some of the leaves 15 hours after inoculation. Infection of uninjured, inoculated leaves was observed 24 hours after inoculation. Control plants were free of the disease 36 hours after inoculation.

These experiments show that bacterial soft rot infection of spinach leaves may take place either through injured or uninjured tissue. Infection apparently occurs at a more rapid rate through wounds. Observations throughout the course of the experiments indicated that the spinach and the *E. carotovora* isolates were equally virulent to spinach.

In order to obtain more information on the effect of the various temperatures on the incidence of soft rot of spinach, leaves were washed in tap and distilled water. One lot was atomized with a water suspension of one of the spinach isolates, one lot with a suspension of *E. carotovora*, and a third lot with distilled water. The various lots were placed in damp chambers which were, in turn, placed in refrigerators where temperatures of 4.5°, 7.5° and 10.0° C. were constantly maintained. At the end of 5 days no rot had developed in the lots held at 4.5° and 7.5° C. During this period one leaf in the damp chamber held at 10.0° C. became infected. After 8 days the inoculated spinach held at 4.5° was free of infection, one leaf in the damp chamber held at 7.5° was infected, while all the leaves in the damp chamber held at 10° were rotted. Control leaves remained uninfected.

These data indicate that during a 5 to 8 days' transit period the development of spinach soft rot would be very materially checked if a temperature of 4.5° C. was maintained. This is borne out by the Fresh Products Inspection Division records, which show that the incidence of spinach soft rot is often held to one per cent or less if the temperature in transit does not exceed 4.5° C.

Further inoculation tests on potato tubers with the spinach and the *E. carotovora* isolates showed that all were pathogenic to this host. It was noted that the rot induced by the *E. carotovora* isolates occurred at a more rapid rate and was more extensive than that caused by the spinach isolates.

SUMMARY

Bacterial soft rot of harvested spinach is described. Morphological characteristics of the cells of spinach soft rot isolates and of *Erwinia carotovora* cultures were not sufficiently distinct to separate them one from another. Results of cultural and biochemical tests indicate that the spinach and the *E. carotovora* isolates are closely related if not identical. Differences in carbon metabolism are not considered sufficient to differentiate the spinach and the *E. carotovora* isolates. Pathogenicity experiments show that bacterial soft rot of spinach may take place through injured or uninjured leaves. Isolates from rotted spinach proved pathogenic to potato tubers. Spinach soft rot may be controlled in transportation and marketing if a temperature of 4.5° C. is maintained for 8 days.

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EXPERIMENTS WITH PEA SEED TREATMENTS IN COLORADO[1]

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INTRODUCTION

Increasing difficulty has been encountered in obtaining satisfactory stands in some of the older pea-growing sections of Colorado. Although the effects of pea seed treatments have been reported from other states (4, 5, 6, 8, 9, 11, 12), conditions under which peas are grown in Colorado differ from those of most other pea-growing sections. In fact, the pea-growing regions within the state are extremely variable as to soils, altitude, and climate. Colorado soils vary from adobe and adobe-like aggregates through sand and sandy-loam to gravel. Altitudes of the pea-growing sections vary from 4,200 feet at Rocky Ford to 7,600 feet in the San Luis Valley. Lower temperatures are associated with the higher altitudes. Cultural practices also vary somewhat, subirrigation being used in the San Luis Valley and row irrigation being practiced in the rest of the state. Since it was not known whether seed treatment materials used in other states would have beneficial effects in Colorado, a series of experiments was designed to determine the effect of fungicidal seed treatments on the emergence and growth of peas under Colorado conditions.

EXPERIMENTAL METHODS

During the summer of 1943, twelve experimental plots of peas were planted in the principal pea-growing areas of Colorado: the northern section, the San Luis Valley, and the Arkansas Valley. Four seed treatments and an untreated control were used in all tests. The chemicals used and the rates of application were New Improved Ceresan (5 per cent ethyl mercury phosphate) at 1 ounce per bushel of seed, and Spergon (98 per cent tetrachloroparabenzoquinone), Arasan (50 per cent tetramethylthiuramdisulfide), and yellow Cuprocide (93 per cent yellow cuprous oxide), each at 2 ounces per bushel.

One plot of each of the four canning varieties, Alaska, Perfection, Wisconsin Sweet, and Green Admiral was planted in the Loveland-Berthoud district. Variety Rogers' 95 was used in three plots in the San Luis Valley,

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[5] The writers wish to acknowledge the cooperation of the Kuner-Empson Canning Co., who furnished the seed of the canning varieties, the growers who furnished land for some of the plots, the United States Rubber Co., who furnished Spergon, DuPont Semesan Co., who supplied New Improved Ceresan and Arasan, Rohm and Haas Co., who furnished Cuprocide, and various members of the staffs of the Colorado Agricultural Experiment Station and Extension Service who assisted in certain phases of the work.

one plot at Rocky Ford, and one at Fort Collins. Variety Little Marvel was used in three plots in commercial market gardens near Fort Collins.

The plot at Rocky Ford, one plot in the San Luis Valley, and one at Fort Collins were planted by hand, but all others were planted with a garden drill. All plots were designed as either Latin squares or randomized blocks, and data obtained were analyzed by the analysis of variance method.

In the Little Marvel tests, 50 pounds of seed were divided into 5 lots of 10 pounds each. Four of these lots were treated and the fifth lot was left untreated. Planting was done with a tractor-drawn pea drill. Stand counts were made by marking off sample strips at each end and in the center of the field.

Stand counts were made for all tests. Yield records were obtained from five of the tests. Living and dead plants were counted on two of the plots at picking time and the percentage of living plants determined. To analyze these results the percentages were converted into degrees of an angle ($\sin^2 \Theta$) by use of Bliss' tables (1, 2).

EFFECTS ON EMERGENCE

Northern Colorado.—No significant stand increases were obtained from any of the seed treatments in the Berthoud district (Table 1). Spergon treatment resulted in a highly significant[6] stand increase over that from untreated or Cuprocide-treated seed of variety Wisconsin Sweet in a test near Loveland. In this same test stands from New Improved Ceresan and Arasan treatments were significantly[6] greater than those from untreated or Cuprocide-treated seed. In the variety Green Admiral, near Loveland, all treatments resulted in highly significant increases in stand. The stand from Spergon-treated seed was significantly better than that from any other treatment.

In plot 5 in the Fort Collins area Little Marvel seed treated with New Improved Ceresan or Arasan gave stands which were significantly better at the 1 per cent level than stands from untreated seed. Cuprocide was better at the 5 per cent level. The stand from New Improved Ceresan was significantly better than that from Spergon or Cuprocide. In a second planting of this variety (Plot 6) stand increases which were highly significant resulted from all treatments. New Improved Ceresan, Spergon, and Arasan produced stands which were significantly better at the 1 per cent level than Cuprocide. In a third planting (Plot 7) Spergon and Cuprocide gave significant stand increases over the untreated.

In the plot of Rogers' 95 at Fort Collins, Spergon, Arasan, and Cuprocide treatments all gave stand increases which were highly significant. New Improved Ceresan was significantly better than the untreated. The stand from Spergon treatment was better than the stand from New Improved Ceresan at the 5 per cent level of significance.

[6] "Highly significant" as used in this paper refers to the 1 per cent level of significance; "significant" refers to the 5 per cent level of significance.

TABLE 1.—*Effect of seed treatment on emergence*

Plot No.	Variety	Location	Mean numbers of plants emerged per plot					Diff. req. for sig. 5% level	Diff. req. for sig. 1% level
			Untreated	Spergon	Arasan	Cuprocide	New Improved Ceresan		
1	Alaska	Berthoud	649.6	675.4	641.2	604.6	626.4	N.S.[c]	N.S.
2	Perfection	Berthoud	976.6	1016.0	829.8	738.0	931.8	N.S.	N.S.
3	Wisconsin Sweet	Loveland	580.6	689.0	657.8	555.6	677.2	74.3	104.1
4	Green Admiral	Loveland	351.8	528.6	476.2	487.2[b]	483.4	41.0	57.5
5	Little Marvel Planted April 16	Ft. Collins	189.3	207.6	227.0	217.3[b]	244.3	25.3	36.8
6	Little Marvel Planted May 1	Ft. Collins	460.6	513.6	507.6	486.0[b]	516.3	12.0	17.5
7	Little Marvel Planted June 8	Ft. Collins	333.6	407.0	364.3	394.6[b]	370.0	56.0	81.5
8	Rogers' 95[a]	Ft. Collins	144.8	190.8	186.2	182.2	166.6	20.2	27.9
9	Rogers' 95[a]	Rocky Ford	34.6	76.8	79.0	82.0	88.8	10.7	15.0
10	Rogers' 95	Center	98.0	128.8	121.8	101.2	162.0	22.6	31.6
11	Rogers' 95[a]	Center	130.6	183.4	182.4	196.8	184.6	14.1	19.7
12	Rogers' 95	Sanford	16.0	61.2	26.0	47.4	49.6	22.7	31.8

[a] Planted by hand. All others were drilled.
[b] Graphite mixed with Cuprocide.
[c] N.S. = not significant.

Arkansas Valley.—At Rocky Ford all seed treatments resulted in stand increases which were highly significant. The increase from New Improved Ceresan was significantly better than the increase from Spergon, but not significantly better than increases from Arasan or Cuprocide.

San Luis Valley.—Highly significant stand increases were obtained from all treatments in the hand-planted plot 11 on the San Luis Valley Demonstration Farm near Center. In the drilled plot 10, New Improved Ceresan was better than any other treatment at the 1 per cent level. Spergon and Arasan were significantly better than no treatment.

Although all stands were poor on the Sanford plots, Spergon and New Improved Ceresan produced stands that were better at the 1 per cent level and Cuprocide produced stands that were better at the 5 per cent level than those of the untreated seed. Spergon was superior to Arasan at the 1 per cent level.

EFFECTS ON YIELD AND SURVIVAL OF PLANTS

No significant differences in yield were obtained in the Alaska variety at Berthoud (Table 2). In Green Admiral the increase from Spergon over all other treatments was highly significant.

The yields of Little Marvel represent the total yield of pod peas obtained from the 10 pounds of seed for each treatment. This was not a replicated plot so data could not be analyzed statistically. However, the field selected for this test was uniform so it seems unlikely that the results were due to chance. Increases over yields from untreated seed were: New Improved Ceresan 22.2 per cent, Cuprocide 18.4 per cent, Arasan 17.6 per cent, Spergon 16.6 per cent.

Yield differences were not significant in the San Luis Valley or in the Rogers' 95 trial at Fort Collins. However, increases in yield from New Improved Ceresan-treated seed approached significance in both plots on the Demonstration Farm at Center.

Percentages of living plants at picking time, based on original stand, are shown in table 3. Differences were not significant in either plot.

DISCUSSION

There are two phases to consider in dealing with root-rotting fungi. The first is the rotting of the seed or the destruction of the young seedling before it emerges from the ground. The second phase is the attack made by the parasites on the older plants. These tests show that seed treatment will generally control the first phase. That this has an indirect effect on the second phase is shown by the counts of living plants at harvest time. These counts show that the same percentage of plants live to reach maturity, regardless of seed treatment. This indicates that seed treatment is effective only during the early life of the plants. However, a better stand obtained by treating the seed will result in a greater number of plants reaching maturity.

TABLE 2.—*Effect of seed treatment on yield*

Plot No.	Variety	Location	Mean yield[a] per plot					Diff. req. for sig. 5% level	Diff. req. for sig. 1% level
			Untreated	Spergon	Arasan	Cuprocide	New Improved Ceresan		
1	Alaska	Berthoud	28.2	29.4	28.6	29.4	27.2	N.S.	N.S.
4	Green Admiral	Loveland	24.3	33.7	23.3	26.0	22.0	2.1	2.9
10	Rogers' 95	Center	229.2	219.0	249.2	233.2	335.6	110.9	155.5
11	Rogers' 95	Center	444.2	482.8	420.2	506.6	523.4	81.0	113.6
8	Rogers' 95	Ft. Collins	121.6	132.8	135.6	141.4	117.6	29.8	41.1
5	Little Marvel	Ft. Collins	607.0	708.0	714.0	719.0	742.0	.....	.....

[a] Yields in tests 1 and 4 are in ounces of shelled peas; in tests 10, 11, and 8, in ounces of pod peas; and in test 5, in total pounds of pod peas.

TABLE 3.—*Effect of seed treatment on survival of plants*

Plot No.	Variety	Location	Per cent of living plants at picking time[a]					
				Untreated	Spergon	Arasan	Cuprocide	New Improved Ceresan
8	Rogers' 95	Ft. Collins	Actual per cent	60.64	66.46	56.24	66.30	50.90
			Converted value	51.43	54.71	48.68	54.62	45.52
11	Rogers' 95	Center	Actual per cent	66.98	59.34	59.82	64.64	63.40
			Converted value	52.87	50.53	50.75	53.64	52.87

[a] Differences are not significant.

In these experiments the same seed treatment did not give similar results under all conditions. This has also been observed by workers in other states (7, 8, 10, 11, 13). The inconsistency may be attributed to a number of factors. Jones (7) showed that variations in soil moisture near the time of planting and differences in soil temperatures cause wide variations in the results obtained from seed treatment. McNew (10) points out that different seed lots vary greatly in their ability to withstand seed decay. Experiments at the Colorado Agricultural Experiment Station indicated that seed treatments vary in their effectiveness against different fungi (3).

There is a great deal of variation in Colorado soils. The soils in which the tests were conducted varied as to type, fertility, moisture content at planting time, and fungus infestation. Isolations from diseased plants showed that several pathogenic fungi were present in some of the soils. While no attempt was made to determine the relative distribution of these fungi, *Fusarium solani* (Mart.) v. *martii* (App. et Wr.) Wr. f. 2 Sny. was obtained most frequently in the isolations. Other pathogens isolated were *Ascochyta pinodella* Jones, *Pythium* sp., and *Corticium vagum* B. et C. var. *solani* Burt. (*Rhizoctonia solani* Kuhn.).

Spergon gave the greatest increase in stand in seven of the tests, New Improved Ceresan was first four times, and Yellow Cuprocide led in one trial. In no case was the untreated lot superior. From tests in New York State McNew (8) concluded that New Improved Ceresan should not be used on peas because it was too injurious when applied at 1 ounce per bushel of seed. Our trials in Colorado do not substantiate this, since New Improved Ceresan gave the best results in some trials.

In the trial with greatest increase in stand from Cuprocide the seed was planted by hand. During planting of the other plots it was noted that Cuprocide-treated seed tended to bind in the drill, so that many of the seeds were cracked. Addition of graphite seemed to eliminate this trouble. This may explain why the stand counts were low in some of the Cuprocide-treated plots when graphite was not used.

While these results are by no means conclusive as to the superiority of one treatment over another, they do show that in most cases any of the materials tested gave better stands than no treatment. Work to determine more of the factors which influence the effectiveness of seed treatments is necessary before complete recommendations can be made for any specific locality.

SUMMARY

Pea seed treatments with Spergon, New Improved Ceresan, Arasan, and Yellow Cuprocide in three pea-growing sections of Colorado generally resulted in increased stands. Although Spergon and New Improved Ceresan gave the best results in the majority of the tests, increases in stand and yield varied in the different trials, so that no one treatment was consistently outstanding.

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7. ———. Factors influencing the effectiveness of organic mercury dusts in pea-seed treatment. Jour. Agr. Res. [U.S.] **42**: 25–33. 1931.
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9. ———. Relative effectiveness of organic and inorganic fungicides as seed protectants. (Abstr.) Phytopath. **33**: 9. 1943.
10. ———. Which varieties of peas need treatment? The Canner **96**(12): 14–16, 30, 32, 35. 1943.
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PHYTOPATHOLOGICAL NOTES

Effect of Penicillin on a Plant Pathogen.—Penicillin, whose bacteriostatic properties were first recognized by Fleming,[1] has of late excited considerable interest among pathologists dealing with bacterial diseases of the human body. The product has been described as particularly active against numerous bacterial animal pathogens that are gram-positive. No reference to experiments with plant pathogens has been found by the authors of this note. Because we have been particularly interested in a gram-positive bacterial plant pathogen that is extensively distributed in this region and since we have sought to try anything that promises a degree of control, penicillin was included in the program of experiments.

The first problem in connection with the use of penicillin was that of procuring the substance. Cultures of the penicillin-producing fungus, *Penicillium notatum*, were obtained and grown in sterile, large but shallow, glass vessels. Later a modification of the apparatus described by Clifton,[2] very much like that used in the manufacture of vinegar, was set up. So much of the penicillin-containing liquid was thus made that a considerable quantity that would otherwise have been wasted has been made available for medical use. Media used were a modified Czapek-Dox[3] and corn-grain decoction made by boiling approximately 50 grams of the dry grains in 1 liter of water.

The plant pathogen subjected to the action of penicillin was the gram-positive bacterium, *Erwinia carnegieana* Standring,[4] that has caused extensive destruction of the giant cactus in Arizona and Mexico. Petri-dish cultures were seeded by growing the organism in broth, pouring the latter on sterile agar plates, and draining off the excess broth suspension. Two hours later a small glass penicillin cylinder, approximately 9.5 mm. in length and 5 mm. in diameter, was seated in the center of the Petri-dish culture, following the procedure described by Abraham *et al.*[3] For comparison a similar series of plates was set up for *Staphylococcus aureus*, the bacterium that is used as a test in experiments with the penicillin drug.

Penicillin suppresses the cactus plant pathogen as it does *Staphylococcus aureus* (Fig. 1). The clear zone around the penicillin chamber in the culture of *Staph. aureus* is even smaller than that in the culture of the plant pathogen. However, the cultures were grown at room temperature, which is more favorable for the development of the plant pathogen.

Besides the results with *Erwinia carnegieana*, we have indications that the gram-positive *Corynebacterium sepedonicum* likewise is susceptible to

[1] Fleming, Alexander. On antibacterial action of cultures of *Penicillium*, with special reference to their use in isolation of *B. influenzae*. British Jour. Exp. Path. **10**: 226–236. 1929.

[2] Clifton, C. E. Large-scale production of penicillin. Science **98**: 67–70. 1943.

[3] Abraham, D. L., A. D. Gardner, E. Chain, N. G. Heatley, C. M. Fletcher, M. A. Jennings, and H. W. Florey. Further observations on penicillin. Lancet **241**: 177–188. 1941.

[4] Lightle, Paul C., Elizabeth T. Standring, and J. G. Brown. A bacterial necrosis of the giant cactus. Phytopath. **32**: 303–313. 1942.

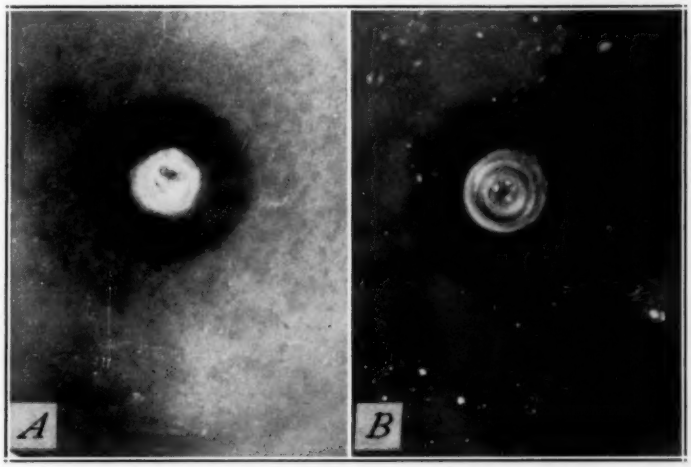

FIG. 1. Effect of penicillin on (A) *Staphylococcus aureus* and (B) *Erwinia carnegieana*. The penicillin-containing liquid was placed in the small glass cylinders after the plates were seeded. Diffusion of the penicillin into the agar suppressed bacterial growth in the circular "clear zones."

the action of penicillin. The bacterial-ring-rot pathogen is slower growing and somewhat more difficult to work with on that account.—J. G. BROWN and ALICE M. BOYLE, University of Arizona, Tucson, Ariz.

Improved Cork-borer Method for Inoculating Trees.—The writer has used the previously described cork-borer method for inoculating trees[1] and has found it satisfactory when inoculating small numbers of trees. During the summer of 1943 approximately 1800 chestnut hybrids were inoculated at from one to three points on each tree, resulting in approximately 3300 inoculations. In large-scale inoculation work the elimination of even one movement means a considerable saving in time; therefore, the writer devised an improved instrument. When using the ordinary cork borer it is necessary to pick up the accessory wire or rod plunger to punch out the cork plug. When using the improved instrument the thumb of the hand that holds the instrument presses against the plunger, which is an integral part of the borer. The movement, otherwise made to reach for a detached plunger, is now used to pick up the inoculating needle or other item, or is eliminated.

Figure 1, A, shows the improved instrument assembled; figure 1, B, shows the instrument disassembled. To construct the improved device, first force off the cross bar of the cork borer; heat the upper end of the tube to redness and hammer the end lightly to form an inner lip or shoulder. Replace the cross bar on the tube at a point about 1½ inches from the upper end of the tube; braze or solder securely to the tube.

The plunger may be made from copper, aluminum, or plastic rod, of slightly less diameter than the inner bore of the tube. The upper end of the

[1] Wright, Ernest. A cork-borer method for inoculating trees. Phytopath. 23: 487–488. 1933.

plunger for a distance of about $\frac{5}{8}$ inch should be of a smaller diameter to allow passage through the flanged opening in the upper end of the tube. The writer found that an inoculating needle holder of the Rosenberger and Greenman type could be adapted as a plunger with a minimum of effort for a $\frac{1}{4}$ inch cork borer. The aluminum rod of this holder is cut off at about $\frac{5}{8}$ inch from the plastic handle; the end of the rod is bored and tapped to take a machine screw, which holds the coiled spring in place.

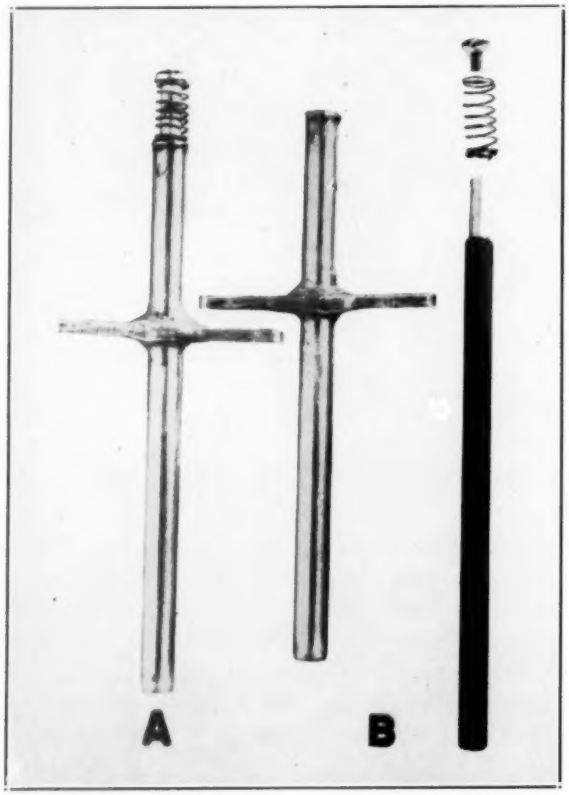

FIG. 1. A. Modified cork borer assembled. B. Modified cork borer disassembled, showing at right the plunger with spring and machine screw detached.

Some pathologists prefer not only to make uniform incisions when inoculating, but also to use uniform amounts of inoculum. A cork borer that is one or two sizes smaller than the borer that makes the incision can be used to cut plugs of inoculum in a Petri dish. The plugs may be cut in the laboratory so that they will be ready to be lifted out with the inoculating needle. The inoculum should be stiff for best results.—RUSSELL B. CLAPPER, Division of Forest Pathology, Bureau of Plant Industry, Soils, and Agricultural Engineering, Agricultural Research Administration, U. S. Department of Agriculture, Beltsville, Maryland.

Lime in the Post-Arsenical Sprays as a Means of Reducing Arsenical Injury to Peaches.[1]—Several workers have investigated the effects of calcium hydroxide, calcium carbonate, and mixtures of these two compounds on the formation of soluble arsenic from lead arsenate in a water solvent. It has been shown that calcium hydroxide is very effective in reducing the concentration of water-soluble arsenic, that calcium carbonate increases the formation of soluble arsenic compared with that of lead arsenate alone,[2] and that combinations of calcium hydroxide and calcium carbonate are effective in

TABLE 1.—*Effect of a delayed lime application on the development of arsenical injury to peach foliage*

Treatment no.	Materials[a] used at shuck split and first cover applications	Materials[a] used at second cover applications	Foliage injury			
			Golden Jubilee variety[b]		Fireglow variety	
			Defoliation	Remaining leaves with necrotic areas from spray injury	Defoliation	Remaining leaves with necrotic areas from spray injury
			Per cent	*Per cent*	*Per cent*	*Per cent*
1A	Acid lead arsenate 2 lbs., sulphur 8 lbs., per 100 gals.	Sulphur 8 lbs., lime 8 lbs.	15.0	35.0	5.0	10.0
1B	do	Sulphur 8 lbs.	75.0	100.0	30.0	90.0
2A	Acid lead arsenate 2 lbs., hydrated lime 6 lbs., sulphur 8 lbs.	Sulphur 8 lbs., lime 8 lbs.	3.0	7.0	1.0	5.0
2B	do	Sulphur 8 lbs.	27.0	71.0	15.6	67.5
3A	Acid lead arsenate 2 lbs., hydrated lime 16 lbs., sulphur 8 lbs.	Sulphur 8 lbs., lime 8 lbs.	0.0	Trace	Trace	Trace
3B	do	Sulphur 8 lbs.	0.0	0.0	Trace	3.3

[a] Magnetic Brand Sulphur (325-mesh) used in all cases.

[b] The foliage on the Golden Jubilee trees in the 1B and 2B treated blocks was lighter green than trees in the corresponding A blocks.

reducing the formation of soluble arsenic from lead arsenate.[3] This last statement is made because Van der Meulen and Van Leeuwen report that "... carbonation takes place very slowly, and ... as long as there is any unchanged calcium hydroxide the amount of soluble arsenic is very low."

Arsenical injury to peach foliage and twigs in New Jersey often has been observed to appear a rather long time (as much as one month) after the application of the last arsenical spray. It seemed probable that this delayed

[1] Journal Series paper of the New Jersey Agricultural Experiment Station, Rutgers University, Department of Plant Pathology.

[2] Ginsburg, Joseph M. Chemical effect on lead arsenate of certain salts which may be present in soil and spray waters. Jour. Agr. Res. [U.S.] 60: 199–205. 1940.

[3] Van der Meulen, P. A., and E. R. Van Leeuwen. A study of lead arsenate and lime spray mixtures. Jour. Agr. Res. [U.S.] 35: 313–321. 1927.

arsenical injury was due either to the carbonation of the lime present or to the removal of the calcium hydroxide by weathering. To test the value of adding lime to the post-arsenical sprays as a means of preventing the occurrence of late-developing injury, three tests were made during the growing season of 1943.

In tests 1 and 2, peach trees were sprayed with mixtures containing sulphur (8 lbs.), acid lead arsenate (2 lbs.), and various concentrations of high-calcium hydrated lime (0, 6, and 16 lbs. per 100 gals.) at two consecutive spray periods. Sprays were applied at the usual times, those of the shuck split and first cover applications. In the third test, one application of lead arsenate, sulphur, and lime was made about 6 weeks after shuck split.

In all tests, each group of similarly treated trees was divided, after application of the sprays containing lead arsenate, into two lots, one lot receiving a sulphur-hydrated lime spray, and the other sulphur alone. This application of sulphur and lime or sulphur alone followed the last lead arsenate spray by 12 to 19 days.

Test 1 was in southern New Jersey on J. H. Hale and Elberta peach trees. Environmental conditions were not conducive to arsenical injury, and consequently no advantage was noted from the use of the delayed lime application. Arsenical injury was not pronounced in any treatment, and the amount of injury did not appear to increase in any block after the delayed lime application.

Test 2 was in a block of Golden Jubilee trees in New Brunswick where environmental conditions, during the test, were conducive to the development of arsenical injury. The data (Table 1) were taken two weeks after the last spray was applied.

Test 3 was in New Brunswick on trees of the Fireglow variety. When the delayed lime spray was applied, foliage injury was already visible. The data (Table 1) were obtained by making foliage counts 18 days after the last spray was applied.

From the results of these experiments, it is apparent that the inclusion of hydrated lime in a spray following by approximately two weeks one containing lead arsenate may materially reduce the amount of foliage injury to peaches that results from the use of lead arsenate.—ROBERT H. DAINES, New Jersey Agricultural Experiment Station, New Brunswick, N. J.

A Method of Inoculating Peach Seedlings with Crown Gall without Using Punctures.—The host range of crown gall has largely been determined either by making artificial injuries and inserting the crown-gall organism from pure cultures, or by growing the host in infected soil. The results presented in this paper show a water suspension from uninjured galls to be an efficient means of placing the crown-gall organism in the soil in contact with peach seedlings that were not artificially injured. Banfield[1] has shown that unin-

[1] Banfield, W. M. Life history of the crown-gall organism in relation to its pathogenesis on the red raspberry. Jour. Agr. Res. [U.S.] 48: 761–787. 1939.

jured crown gall on raspberries can liberate in water large numbers of the crown-gall organisms. Smith and Cochran[2] have reported that a high percentage of crown gall occurs on stone fruits in nurseries on land having no previous history of crown gall, and have associated this trouble with irrigation water that was contaminated with the crown-gall organism.

To test this avenue of infection, peach pits were planted in a dark-colored mountain potting soil (pH 8.36) that had previously been treated with chloropicrin gas. Twenty-five peach seeds that were just beginning to germinate, but were not sufficiently advanced to be injured in handling, were planted in each 5-gallon container. One week after planting, at time of emergence, the soil in some of the containers was watered with a suspension obtained from soaking 20 good-sized crown galls in 20 quarts of water over night. One quart of suspension was applied per can.

The seedlings were dug and results taken in January, after one season's growth. The results can be summarized as follows:

329 trees treated with suspensions—175, or 53 per cent, with galls
93 trees not treated (controls)— 9, or 9.6 per cent, with galls

There were some galls in the nontreated or control trees. These galls cannot be satisfactorily accounted for, but may have resulted from contamination by spattering from the infected soil of inoculated cans, or from the incomplete sterilization of the planting soil, or from the pits stratified in sand and peat. The irrigation water for these trees was from the deep wells of the Riverside city water system. It is well established that injuries are necessary for the entrance of the crown-gall organism into the host. Siegler and Bowman[3] have observed injuries made in germinating peach seedlings near where cotyledons are attached. Other injuries may occur in rapid-growing seedlings where lateral roots arise or where soil insects injure the roots. The soil in these experiments was treated with chloropicrin before being used, and was apparently free of injurious insects. This, however, does not preclude the possibility of their having been introduced with the rinse water from the unsterilized galls used in the experiment.—CLAYTON O. SMITH, University of California Citrus Experiment Station, Riverside, California.

The Term Viruliferous.—The medical term *viruliferous* was derived from *virulentus*, meaning virulent or full of virus, and *fero*, meaning bear or carry, with the idea of motion predominating. This meaning, virus-bearing, was indicated by Carsner and Stahl[1] when they substituted the established medical term *viruliferous* for the term *viriferous*, which they[2] had a little earlier coined. This latter term was so defined as to describe

[2] Smith, C. O., and L. C. Cochran. U. S. Dept. Agr. Bur. Plant Indus., Plant Dis. Reptr. **28**: 160–162. 1944.

[3] Siegler, E. A., and J. J. Bowman. Crown gall of peach in the nursery. Phytopath. **30**: 417–426. 1940.

[1] Carsner, E., and C. F. Stahl. Studies on curly-top disease of the sugar beet. Jour. Agr. Res. [U.S.] **28**: 297–320. 1924.

[2] Stahl, C. F., and E. Carsner. A discussion of *Eutettix tenella* Baker as a carrier of curly-top of sugar beets. Jour. Econ. Ent. **16**: 476–479. 1923.

the status or condition of an insect vector, in this case the beet leafhopper, *Eutettix tenellus* (Baker), in which it carries a virus, in this case the curly-top virus. Leach[3] defines *viruliferous:* "Capable of inducing a disease by feeding on the suscept."

The term *viruliferous* has gradually come into extensive and effective use in plant pathology in the above sense. This precise usage may be corrupted unless divergent practices are avoided. Valleau[4,5] has referred to plant tissue as *viruliferous.* It would follow from this that any plant affected with a virus disease is *viruliferous.* Indeed, Johnson and Jones[6] have adopted such usage. Such corrupting of a precise, highly useful term ought to be discouraged. The Committee on Terminology (Nomenclature) of Immunology and Use of Technical Words of the American Phytopathological Society could help in this situation by recommending an appropriate, restrictive definition.

Frampton, Linn, and Hansing[7] reintroduced the term *viriferous* which they preferred to *viruliferous.* It is to be hoped that few if any other writers will follow their lead in this.—EUBANKS CARSNER, U. S. Department of Agriculture, Riverside, California.

Resistance of Guayule to the Root-knot Nematode.—At the time the emergency rubber project was started by the United States Department of Agriculture, little was known concerning the diseases of guayule, *Parthenium argentatum* Gray. Until this xerophytic plant was removed from its natural habitat and placed under cultivation, the only parasites recorded[1] were a rust, *Puccinia parthenii* (Speg.) Arthur, and dodder, *Cuscuta* sp. Recently, various workers[2, 3, 4, 5, 6, 7, 8] have given attention to certain root diseases in nurseries and experimental plantings. Among the root diseases

[3] Leach, J. G. Insect Transmission of Plant Disease. 615 pp. New York. 1940.

[4] Valleau, W. D. Experimental production of symptoms in so-called recovered ring-spot tobacco plants and its bearing on acquired immunity. Phytopath. 31: 522–533. 1941.

[5] Valleau, W. D. Control of the common mosaic disease of tobacco by breeding. Phytopath. 32: 1022–1025. 1942.

[6] Johnson, F., and L. K. Jones. A report on a study of virus transmission by fungi and nodule bacteria of peas. U. S. Dept. Agr., Plant Disease Reporter 27: 656–658. 1943.

[7] Frampton, V. L., M. B. Linn, and E. D. Hansing. The spread of virus diseases of the yellows type under field conditions. Phytopath. 32: 799–808. 1942.

[1] Lloyd, Francis Ernest. Guayule (*Parthenium argentatum* Gray), a rubber-plant of the Chihuahuan Desert. Carnegie Inst. Wash. Publ. 139. 1911.

[2] Campbell, W. W., L. D. Leach, J. T. Presley, and W. C. Snyder. Some diseases of guayule in California. U. S. Dept. Agr. Plant Dis. Rptr. 27: 63–66. 1943.

[3] Ezekiel, Walter N. Crown rot and root rot of guayule. U. S. Dept. Agr. Plant Dis. Rptr. 27: 2–8. 1943.

[4] Hoyman, Wm. G. Preliminary evidence suggests guayule may be resistant to the root-knot nematode. U. S. Dept. Agr. Plant Dis. Rptr. 26: 476. 1942.

[5] Presley, John T. Some diseases affecting cultivated guayule in the Southwest during 1942. U. S. Dept. Agr. Plant Dis. Rptr. 27: 94–96. 1943.

[6] Streets, R. B. The susceptibility of guayule to Phymatotrichum root rot. U. S. Dept. Agr. Plant Dis. Rptr. 27: 66–68. 1943.

[7] Taubenhaus, J. J., and Walter N. Ezekiel. A rating of plants with reference to their relative resistance or susceptibility to Phymatotrichum root rot. Texas Agr. Exp. Sta. Bull. 527. 1936.

[8] Watkins, G. M. Diseases of guayule observed in Texas. U. S. Dept. Agr. Plant Dis. Rptr. 27: 591. 1943.

mentioned, it is interesting to note that guayule is not regarded as being very susceptible to *Phymatotrichum omnivorum* (Shear) Duggar, a fungus indigenous to the Southwest where guayule is being planted. Taubenhaus and Ezekiel[7] list guayule as being moderately susceptible while Streets[6] has reported only a trace of infection in the first year's growth under irrigation. Presley[5] was unable to produce infection under laboratory or field conditions.

At some locations where guayule is being planted it is quite probable that the root-knot nematode, *Heterodera marioni* (Cornu) Goodey, occurs. Campbell, Leach, Presley and Snyder[2] have observed that plants in the older nursery beds at Salinas, California, showed light infestations. In an experiment in 1942 at Tucson, Arizona, 13 transplants[9] remained free from infestation after growing in the field in infested soil from March 20 until October. Six other transplants were grown six months in pots containing naturally infested soil. When the soil was removed from their roots, only a very slight infestation was found on one plant. The roots and tops of these six plants were pruned and returned to the original pots, and through the winter months the plants were placed in the greenhouse where the temperature was never below 20° C. Tomatoes were seeded with the six guayule plants to serve as nematode indicators. When the tomato seedlings were removed, the roots were severely infested with the root-knot nematode[10] indicating that the soil was heavily infested. Twelve months after the guayule had been returned to the pots, the roots were examined for infestation but none was apparent. The soil was returned to the pots and tomatoes were again seeded. The amount of infestation on the seedling roots indicated *Heterodera marioni* was still present in sufficient numbers to cause severe infestation of this indicator plant.

Additional evidence as to the resistance of guayule was obtained from 10 transplants growing in pots containing artificially infested, sandy soil. This experiment was started in October, 1942, in the greenhouse where the temperature was 20° C. or higher. Tomatoes were seeded in October in the pots containing the guayule transplants and when removed as seedlings the roots were severely infested with the root-knot nematode. In October, 1943, the guayule roots were removed from the pots and only a very slight infestation was observed on one of the 10 plants. The soil was returned to the pots and tomatoes were again seeded. The severe infestation observed on the seedling roots was evidence that the nematode population was still very great. These data indicate that guayule is very resistant to the root-knot nematode.—WM. G. HOYMAN, Department of Plant Pathology, University of Arizona, Tucson, Arizona.

[9] The guayule transplants used in this investigation were obtained from one of the Forest Service Nurseries, Salinas, California.

[10] The root-knot nematodes occurring in the infested tomato roots were identified by Gerald Thorne, Associate Nematologist, United States Department of Agriculture, Salt Lake City, Utah.

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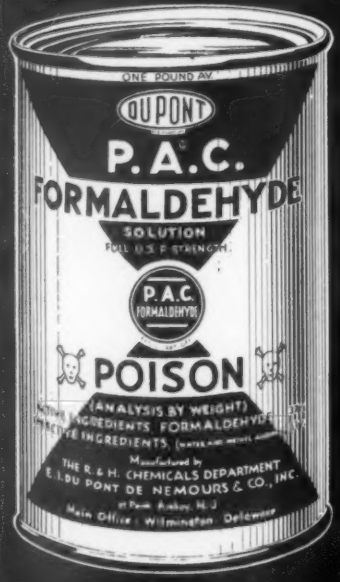

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